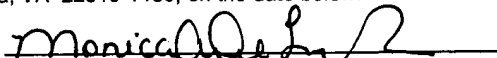


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November 9, 2004

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Monica A. De La Paz

**PATENT**

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:  
Wold *et al.*

Serial No.: 09/351,778

Filed: July 12, 1999

For: Replication Competent Anti-Cancer Vectors

Group Art Unit: 1632

Examiner: Priebe, Scott David

Atty. Dkt. No.: 66153-7775

**SUPPLEMENTAL BRIEF ON APPEAL**

**MS Appeal Brief-Patents**  
Commissioner for Patents  
P.O. Box 1450  
Alexandria, VA 22313-1450

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APPENDIX 1: Claims Appendix

APPENDIX 2: Evidence Appendix



Appellants hereby submit an original and three copies of this Supplemental Appeal Brief to the Board of Patent Appeals and Interferences in response to the Notification of Non-Compliance with 37 C.F.R. §1.192(c), dated September 9, 2004 and the final Office Action. This Supplemental Appeal Brief incorporates format changes in accordance with the new rules changes for Appeal Briefs set forth in the Federal Register. See Federal Register, 69(155):50005-50007, 2004.

The Notice of Appeal was filed on May 20, 2004, and received in the Patent and Trademark Office on May 24, 2004. The Appeal Brief was filed on July 28, 2004. A Supplemental Appeal Brief that did not include the new format changes was submitted Nov. 3, 2004. The initial deadline for re-filing the revised Appeal Brief was October 9, 2004. A fee for a one-month extension of time was included with the Supplemental Appeal Brief dated Nov. 3, 2004. Thus, this brief is timely filed and no additional fee is due.

If the check submitted with the Supplemental Appeal Brief dated Nov. 3, 2004 is inadvertently omitted, or the amount is insufficient, or should any additional fees under 37 C.F.R. §§ 1.16 to 1.21 be required for any reason relating to the enclosed materials, or should an overpayment be included herein, the Commissioner is authorized to deduct or credit said fees from or to Fulbright & Jaworski L.L.P. Account No.: 50-1212/INGN:109US.

Please date stamp and return the attached postcard as evidence of receipt.

#### **I. REAL PARTIES IN INTEREST**

The real parties in interest are St. Louis University (assignee), VirRx (licensee), and Introgen Therapeutics, Inc. (sublicensee).

#### **II. RELATED APPEALS AND INTERFERENCES**

There are currently no related appeals or interferences.

### **III. STATUS OF THE CLAIMS**

The application was filed with original claims 1-27. Claims 28-108 were added during prosecution. Of these, claims 1-4, 10, 45-59, 85-96 have been previously canceled, and claims 6-9, 16-19, 23, 25-31, and 76-84, directed to non-elected species with no currently allowable generic or linking claim, stand withdrawn. Thus, claims 5, 11-15, 20-22, 24, 32-44, 60-75, and 97-108 are pending. Of these, claims 11-15, 20-22, 24, 32-44, 60-75, and 97-108 are subject to the present appeal, with the subject matter of claims 14-15, 24, 62, 63, 67, 70-71 and 100 being free of the prior art. A copy of the appealed claims is attached as Appendix 1.

### **IV. STATUS OF AMENDMENTS**

No amendments were sought to the pending claims following final rejection.

### **V. SUMMARY OF CLAIMED SUBJECT MATTER**

The present invention is directed generally to the use of a special type of replication-competent "oncolytic" adenoviruses in the treatment of cancer. Specification, page 1, lines 8-10; and page 10, line 35 through page 11, line 2.<sup>1</sup> The oncolytic adenoviruses of the present invention are special in terms of their expression of the ADP gene. Specification, page 5, lines 16-31. The ADP gene (which stands for "adenovirus death protein") is the gene discovered by the present inventors to promote oncolysis of cancer cells that are infected with these special adenoviruses. Specification, page 5, lines 16-31. The claims can generally be divided into two categories. One category, the "ADP overexpressing" adenovirus claims, are exemplified by claims 13 and 101, and claims depending therefrom:

13. A method for treating cancer in an animal having a tumor comprising administering to the tumor an adenovirus vector wherein said adenovirus vector is replication-competent in neoplastic cells and overexpresses

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<sup>1</sup> Appellants note that citations to the Specification identify support for the claimed invention. However, such citations in no way constitute the only support, as other support in the Specification can be found and relied upon, if necessary.

an adenovirus death protein (ADP), wherein overexpression is defined as overexpression relative to *dI309*.

101. A method for treating cancer in an animal having a tumor, the method comprising administering to the tumor an adenovirus vector wherein said adenovirus vector is replication-competent in neoplastic cells and overexpresses an adenovirus death protein (ADP).

The second category of claims are not *per se* limited to “ADP overexpression” and are directed to adenoviruses defined in terms of structural instead of functional limitations. This category is exemplified by claim 60 and claims depending therefrom:

60. A method for treating cancer in an animal having a tumor, the method comprising administering to the tumor an adenovirus vector that is replication-competent in neoplastic cells and expresses an adenovirus death protein (ADP), wherein:

- a) the ADP is expressed from an ADP coding sequence positioned under the control of a promoter other than the endogenous promoters for ADP;
- b) the adenovirus vector comprises a deletion in the E3 region that removes a splice site for an E3 mRNA;
- c) the ADP is expressed from an ADP coding sequence flanked by a pre-mRNA splicing and cleavage/polyadenylation signal other than the pre-mRNA splicing and cleavage/polyadenylation signal normally associated with the ADP gene, and/or
- d) the ADP is expressed from an ADP coding sequence that is positioned downstream of the coding sequence for another adenovirus mRNA, together with a sequence on the 5’ side of the ADP coding sequence that allows for internal initiation of translation of ADP.

Lastly, claim 102 specifies ADP overexpression that is achieved by one of the foregoing structural limitations:

102. A method for treating cancer in an animal having a tumor, the method comprising administering to the tumor an adenovirus vector wherein said adenovirus vector is replication-competent in neoplastic cells ***and overexpresses an adenovirus death protein (ADP)***, wherein overexpression is effected by one of more of the following modifications:

- a) the ADP is expressed from an ADP coding sequence positioned under the control of a promoter other than the endogenous promoters for ADP;
- b) the adenovirus vector comprises a deletion in the E3 region that removes a splice site for an E3 mRNA;
- c) the ADP is expressed from an ADP coding sequence flanked by a pre-mRNA splicing and cleavage/polyadenylation signal other than the pre-mRNA splicing and cleavage/polyadenylation signal normally associated with the ADP gene, and/or
- d) the ADP is expressed from an ADP coding sequence that is positioned downstream of the coding sequence for another adenovirus mRNA, together with a sequence on the 5' side of the ADP coding sequence that allows for internal initiation of translation of ADP.

The specification describes a series of exemplary adenoviruses that overexpress ADP, including the KD series (KD1, KD2, KD3) and the GZ series (GZ1, GZ3) and further describes the use of these vectors in the treatment of human cancer as exemplified through accepted animal models [Specification, Examples 1 – 4 (page 20, line 31 through page 29, line 11), FIGS. 1-9]. The KD series include mutations in their E1 regions that appear to limit their replication to cancer cells, whereas the GZ series do not have such limitations [Specification, Examples 1 – 8 (page 20, line 31 through page 40, line 29)]. All of these vectors have demonstrated significant if not surprising antitumor oncolytic efficacy [Specification, Examples 2, 4, 7, and 8, page 25, line 6 through page 37, line 33]. One such vector, designated INGN007 (i.e., GZ3), is currently in preclinical development by Introgen Therapeutics, Inc. and is expected to be in the clinic in the very near future.

## **VI. GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL**

This appeal presents the following issues:

- a) Whether the subject matter of the appealed claims is adequately described in the subject specification as required by 35 U.S.C. §112, first paragraph;
- b) Whether the claims 101-102 are indefinite under 35 U.S.C. §112, second paragraph;
- c) Whether the subject matter of claims 10-13, 32-44, 60, 61, 68, 69, 72-75, 97-99 and 101-108 is anticipated by Henderson *et al.* (“Henderson”; Exhibit 1) or Little *et al.* (“Little”; Exhibit 2) under 35 U.S.C. §102(e);
- d) Whether the subject matter of claims 13, 20-22, 60 and 64-66 is obvious under 35 U.S.C. §103 over the combination of Henderson or Little in light of Freytag (Exhibit 3).

## **VII. ARGUMENT**

### **A. Rejection of Claims under 35 U.S.C. §112, 1<sup>st</sup> Paragraph**

In the Final Action, claims 11-15, 20-22, 24, 32-44, 60-75 and 97-108 were rejected on the basis of 35 U.S.C. §112, first paragraph, and raises the following issues:

- a) Whether the subject matter of the appealed claims (except, presumably, claims 107 and 108) is adequately described in the specification with respect to the phrase “in an animal having a tumor”;
- b) Whether the subject matter of claims 11-15, 20-22, 24, 32-44 and 108 is adequately described in the specification with respect to the phrase “wherein overexpression is defined as overexpression relative to *dl309*”;

- c) Whether the specification provides written description support for the language “is detectable by western blot, cell lysis, virus release or by cell spreading assay” found in claims 32 and 103-106; and
- d) Whether the specification describes the possibility of including more than one of the four alternatives (elements a) through d)) for upregulating the expression of the adenovirus death protein (“ADP”) set forth in claim 102, claim 60 and claims depending therefrom (claims 60-75 and 97-100, 102).

Appellants will address each of these rejections separately below.

**1. *The subject matter of the appealed claims (except, presumably, claims 107 and 108) is adequately described in the specification with respect to the phrase “in an animal having a tumor.”***

**a) *The Examiner’s Rejection***

In the Final Action, the Examiner rejected all of the claims (except, presumably, claims 107 and 108) under §112, first paragraph, taking the position that the specification fails to disclose the treatment of an “animal having a tumor.” The Action acknowledges that the specification does provide support for the treatment of humans having a tumor (claims 107 and 108), but argues that it does not provide written description support for the treatment of animals other than humans. The Action makes reference to the myriad of different types of “animals” that would be embraced by the rejected language.

**b) *Remarks***

In response, Appellants contend that the specification would apprise the skilled artisan that it contemplates treatment of animals with tumors. Thus, the Action’s concern with protozoans and sponges would appear to be unfounded. First of all, the specification is not limited to human tumors and, in fact, contemplates the treatment of “tumors of any origin”:

The invention also provides compositions comprising a recombinant vector that overexpresses ADP in an amount effective for promoting death of neoplastic cells and a method comprising administering a therapeutically effective amount of the vector to a neoplastic cell in a patient. It is believed that the compositions and methods of the present invention *are useful for killing neoplastic cells of any origin* and include neoplastic cells comprising tumors as well as metastatic neoplastic cells.

Specification at page 17, lines 16-21 (emphasis supplied). Furthermore, the specification actually sets forth examples of the treatment of animals. This is shown in Examples 4 and 8 of the specification, which demonstrate the treatment of mice having tumors and specifically uses the word “animal” (see specification, page 27, lines 20-21).

The Action’s position appears to rely on its interpretation of the meaning of the term “patients” and equates this term with “human patients.” However, it is certainly true that an animal can be a “patient,” as evidenced by the fact that we have veterinarians and “animal” hospitals.

The Board is therefore requested to overturn the Examiner on this point.

2. *The subject matter of claims 11-15, 20-22, 24, 32-44 and 108 is adequately described in the specification with respect to the phrase “wherein overexpression is defined as overexpression relative to dl309.”*

The Action next takes the position the specification fails to provide written description support for the phrase “wherein overexpression is defined as overexpression relative to dl309,” found in claims 11-15, 20-22, 24, 32-44 and 108. The Examiner’s arguments can be summarized as follows, and the Appellants’ response to each follow:

- a) *That the dl309 adenovirus is not a wild type adenovirus and that there is no evidence that dl309 expresses ADP as high as any previously known adenovirus.*

In response, Appellants note that whether dl309 is a wild type adenovirus or not, or whether it expresses ADP as high as any previously known adenovirus, is irrelevant to the

written description issue. Nevertheless, it is specifically noted that on page 25, lines 28-30, the specification characterizes Ad5, *dl309* and *dl01/07* as “viruses expressing wild-type amounts of ADP.” The Examiner has presented no evidence to call into question the objective truth of this statement. *In re Marzocchi*, 439 F.2d 220, 223-24, 169 USPQ 367, 370 (CCPA 1971).

The issue is whether one of skill in the art would understand from the specification that the inventors contemplated that *dl309* was intended to be a standard against which one can measure overexpression. From a review of the specification, there can be no doubt that *dl309* was intended to be just such a standard. This is evident from a reading of the specification at page 12, lines 18-23 and Example 1. At page 12, lines 18-23, the specification defines “overexpression of ADP” *per se* (as opposed to overexpression relative to *dl309*) to mean that more ADP molecules are made in the infected cell relative to previously known adenoviruses. Example 1 of the specification tests the four exemplary embodiments of the invention, KD1, KD3, GZ1 and GZ3, by comparing their ability to express ADP relative to exemplary previously known adenoviruses, including *dl01/07*, *dl309*, *dl327* and Ad5 (wild type adenovirus). Specification at pages 21-25.

KD1 and KD3 are adenoviral vectors that have the so-called 01/07 mutations in their E1 region that render them replication competent primarily only in neoplastic cells and which further contain deletions in their E3 region that up-regulate ADP expression. GZ1 and GZ3 have the ADP up-regulating E3 mutations, but do not contain the E1 region mutations and thus their replication is not restricted. Contrary to the Examiner’s position in various previous official actions, **ALL** of these vectors, KD1, KD3, GZ1 and GZ3, are representative of the vectors embraced by the claims in that all are replication-competent in neoplastic cells and all have E3 mutations that upregulate ADP expression.



On page 24 of the specification it is also explained that *dl01/07* is the parent vector of KD1 and KD3, except without the ADP-upregulating E3 mutations. Thus, *dl01/07* is replication-restricted to neoplastic cells but do not overexpress ADP. It is also explained that *dl309* is the parent of all of the exemplary vectors of the invention, except that it contains neither the E1 “replication-restriction” mutations nor the ADP up-regulating E3 mutations. Another control vector, *dl327*, is a negative control in that it lacks the ADP gene altogether.

The studies reported on page 24 show that each of the exemplary vectors of the invention overexpress ADP relative to each of the forgoing vectors.

The Examiner’s main contention is that the specification allegedly does not state the specific words that *dl309* is intended to be a control against which the ADP expression of ALL vectors of the invention are to be measured. However, it is submitted that a fair reading of the specification, from the standpoint of one of skill, is that the vectors were employed in the examples – not just example 1, but in generally each of the examples – for the very purpose of demonstrating what was intended by “ADP overexpression.” Why else were these vectors chosen and presented as ADP expression controls and included as such in virtually all of the examples?

- b) That the expression of ADP by dl309 or the KD or GZ vectors was not measured in terms of molecules of ADP per viral genome as per page 12, lines 18-21.***

The issue of whether the specification discloses a calculation of the number of molecules of ADP per viral genome is irrelevant to the issue of written description for the rejected phrase, which does not call for such a measurement. The phrase simply refers to overexpression of ADP and the specification describes a number of ways for determining such overexpression, as evidenced by Example 1.

3. *The specification does provide written description support for the language “is detectable by western blot, cell lysis, virus release or by cell spreading assay” found in claims 32 and 103-106.*

The Examiner’s main argument, it appears, is that these particular assays for ADP expression levels is only found in the examples for measuring ADP expression with respect to the four exemplary vectors, KD1, KD3, GZ1 and GZ3, and that thus these assays are allegedly not described as a general technique for measuring ADP expression from other vectors within the scope of the claims. With respect to cell lysis assay, the specification analyzes the results of that assay by observing that the assay shows that “over-expression of ADP increases the rate of cell lysis.” Page 25, lines 18-21. Similarly, with respect to the virus release assay, the specification shows that the assay is capable of distinguishing between viruses “which overexpress ADP” and those “expressing wild-type amounts of ADP.” Page 25, lines 28-30. Likewise, with respect to the cell spreading assay, the specification uses the assay to measure the ability of viruses to lyse cells and spread to newly infected cells and demonstrates “the potency of ADP in mediating ... virus spread in A549 cells.” Page 26, lines 5-6.

With respect to the cell lysis and cell spreading assays, the Examiner states that the example “does not suggest that either assay is to be used to determine whether ADP is overexpressed by a given adenovirus vector used in the invention.” Action at page 5. This is not true. The specification, at page 25, lines 20-21, specifically states that “ADP is required for efficient cell lysis, and over-expression of ADP increases the rate of cell lysis.” This statement is a generalized observation that is, on its face, clearly not limited to the exemplary vectors that were tested. Furthermore, as explained in the first full paragraph of page 26 (lines 4-13), the cell spreading assay is simply one way of assessing cell lysis (“This result [the cell spreading assay]

attests to the potency of ADP in mediating cell lysis and virus spread in A549 cells [the target cells used in the assay].”)

The Examiner’s reliance on the *Purdue Pharma* case is misplaced. In *Purdue Pharma*, the limitation at issue was the so-called  $C_{\max}/C_{24}$  ratio which was used in the claims in an attempt to define the class of agents that the claim covered. In the specification at issue there, the  $C_{\max}/C_{24}$  ratio was not discussed at all, but merely employed *ex post facto* to characterize two formulations. Nowhere in the patent specification at issue in *Purdue* was it stated that the  $C_{\max}/C_{24}$  ratio was intended to be a distinguishing characteristic, with the court characterizing the  $C_{\max}/C_{24}$  ratio as “...a characteristic that is not discussed even in passing in the disclosure ...”). 56 U.S.P.Q.2d at 1487.

In contrast, the issue here is the “overexpressing” language and how to interpret that language. There is no question that the specification identifies “overexpression” of ADP as a central focus of the invention, and it is identified throughout the specification as a desirable quality in virtually all embodiments. The question is merely how the specification teaches to measure overexpression, and there are four examples of how to measure overexpression given in Example 1. The Example in no way implies that these four methods are only applicable to KD1, KD2, GZ1 and GZ3 – on the contrary, the specification nowhere says that ADP overexpression is only desirable for these four vectors, and the examples merely provide an example as to how one might proceed to measure ADP expression.

4. ***The specification describes the possibility of including more than one of the four alternatives (elements a) through d)) for upregulating the expression of the adenovirus death protein (“ADP”) set forth in claims 60 and claims depending therefrom (claims 60-75 and 97-100, 102).***

The Action next rejects claim 60, alleging that the specification does not provide support for including “more” than one of the indicated adenovirus modifications at a time. Appellants

traverse this rejection as well. The Board's attention is directed to the specification at page 13, lines 2-8, particularly at line 5. Appellants agree with the Action that this excerpt presents these four characteristics as alternatives. However, it also presents these characteristics as cumulative and combinable, particularly in light of the fact that the specification specifically employs the inclusive connector "and" when listing the possible elements. See specification at page 13, line 5.

## **5. Conclusion**

For the reasons outlined above, the Board is requested to overturn the Examiner's positions regarding written description.

### **B. Rejection of Claims Under 35 U.S.C. §112, 2<sup>nd</sup> Paragraph**

The Final Action next rejects claims 101-102 under 35 U.S.C. §112, 2<sup>nd</sup> paragraph, taking the position that the phrase "overexpresses an adenoviral death protein," renders the claims unclear as to the metes and bounds of the claim. The Action queries regarding "overexpresses relative to what?" and also states that a "reasonably broad interpretation of these claims would suggest any adenovirus carrying ADP operatively linked to its native promoters" would meet the limitation of the claims.

In response to the Action's query regarding "overexpression relative to what," Appellants point out that there is a specific definition, at page 12, lines 18-23, for what overexpression is measured against in the context of claims such as claims 101-102 that do not specify the reference virus. Here it is stated that "'overexpresses ADP' means that more ADP molecules are made per viral genome present in a dividing cell infected by the vector than expressed by any previously known recombinant adenoviral vector or AAV in a dividing cell of the same type." As already discussed above, the specification discloses various assays for determining whether more ADP molecules are made per viral genome.

In light of the very clear definition set forth in the specification, the Action's position that a "reasonably broad interpretation of these claims would suggest any adenovirus carrying ADP operatively linked to its native promoters" would meet the limitation of the claims, is not understood. Such an adenovirus would only meet the limitation of the claims if it was shown to express more ADP molecules per viral genome present in a dividing cell infected by the vector than expressed by any previously known recombinant adenoviral vector in a dividing cell of the same type.

For the foregoing reasons, the Board is requested to overturn the Action's rejections in this regard.

**C. Rejection of claims 10-13, 32-44, 60, 61, 68, 69, 72-75, 97-99 and 101-108 is anticipated by Henderson *et al.* ("Henderson"; Exhibit 1) or Little *et al.* ("Little"; Exhibit 2) under 35 U.S.C. §102(e).**

The Final Action next rejects claims 10-13, 32-44, 60, 61, 68, 69, 72-75, 97-99 and 101-108 as anticipated by Henderson *et al.* ("Henderson"; Exhibit 1) or Little *et al.* ("Little"; Exhibit 2) under 35 U.S.C. §102(e). The Action takes the position that Henderson and Little both teach an adenovirus, CN751, that has E3 deletions yet retains the ADP gene and thus would be expected to overexpress ADP.

Appellants note that claim 10 is included in this rejection. However, claim 10 was canceled as per the Amendment dated December 3, 2003. Appellants will therefore assume that the inclusion of claim 10 in this rejection was erroneous.

In responding to the rejection, Appellants will provide separate arguments with respect to various claims and will group the claims accordingly.

**1. The “Overexpresses ADP” Claims 11- 13, 32-44, 101-106, and 108**

Appellants will first address the anticipation rejection with respect to the “overexpressing ADP” claims that have been rejected, which, of those now active, includes claims 11-13, 32-44 and 101-106, and 108.

**a) It is the Examiner’s Burden to Show Anticipation**

The Final Action takes the position that it is the Appellants’ burden to demonstrate that the CN751 vector of Henderson and Little does not overexpress ADP. Appellants disagree. Since this is an anticipation rejection, the Examiner must demonstrate that each and every element of the claims is either explicitly or necessarily disclosed in the allegedly anticipatory art. That has not been done here. Each of the “overexpresses ADP” claims require *overexpression* of ADP and there is no teaching anywhere in either Henderson or Little that the vectors they describe overexpress ADP, as that term is used.

The Examiner likens the present situation to the examination of product-by-process claims, where the burden is sometimes shifted to applicants due to the fact that the PTO does not have the necessary laboratories to carry out processes and compare the product so produced against the prior art. However, the situation here is different. The claims specify a particular feature that must be possessed to be within the scope of the claim, and the Examiner has presented no evidence to suggest that that the prior art has such a property. To shift the burden to the applicant, the examiner must provide evidence to demonstrate that the prior art “necessarily” has the claimed property. *In re Robertson*, 169 F.3d 743, 745 (Fed. Cir. 1999). As noted by the Federal Circuit, “[i]nherency, however, may not be established by probabilities or possibilities. The mere fact that a certain thing may result from a given set of circumstances is not sufficient.” *Id.* at 1269, 20 U.S.P.Q.2d at 1749 (quoting *In re Oelrich*, 666 F.2d 578, 581, 212 U.S.P.Q. 323, 326 (C.C.P.A. 1981)). The Examiner here has not satisfied this burden.

**b) CN751 Does Not Overexpress ADP**

Furthermore, even if the Examiner has shifted the burden to Appellants to demonstrate that the prior art does not have the claimed property, it is noted that Appellants have done so. Both Henderson and Little teach that CN751 expresses about the same amount of ADP as does wild-type adenovirus, and thus cannot be said to overexpress ADP. This can be seen in Henderson, for example, at col. 49, lines 6-8. Here it is stated that CN751 kills cells more efficiently than an ADP-minus control, but about the same as an ADP-positive wild-type control, Rec700. See also Little *et al.* patent at col. 40, lines 24-26. We know from the present specification that cell killing is a good measure of ADP expression. See Example 2. We also know that dl309, the standard against which claim 13 and claims depending therefrom are measured, expresses “wild-type” levels of ADP. Specification at page 25, lines 28-30. Since we know from Henderson and Little that CN751 expresses wild-type levels of ADP, and in light of the fact that the claims require higher ADP expression than dl309 which expresses wild-type levels, it can be concluded that CN751 does not anticipate these claims.

One of the Action’s responses to this argument is that Appellants have allegedly “indicated on the record (response filed 1/10/02, page 7) that CN751 would be expected to overexpress ADP.” Applicant denies that such a representation was made – the statement referred to by the Examiner was a recitation of the pending rejection that failed to fully attribute the statement to the Examiner. In other words, it was a poorly drafted statement that was never intended to make any admission about what the prior art teaches or does not teach, it simply failed to attribute the statement to the Examiner, which was what was intended. In any event, the question is whether Little or Henderson teaches a vector that overexpresses ADP, and the reality is, as explained above, neither reference teaches overexpression of ADP.

The Action also argues that Henderson/Little teach placing ADP under control of a heterologous promoter (tissue specific or viral promoter), or inclusion of multiple copies of ADP, and that the use of such a heterologous promoter “would be expected to result in overexpression compared to *dl309*.” In response to the Examiner being requested to set forth the basis for the conclusion that either of these embodiments would result in overexpression, the Action now states that the instant specification teaches, at page 13, that placing ADP under the control of a heterologous promoter is one means of achieving ADP overexpression. However, this position is insufficient: There are all kinds of heterologous promoters, and Henderson/Little are silent as to what kind of heterologous promoter should be used and is silent as to whether the ultimate objective is to achieve overexpression, or even that overexpression of ADP is a desirable goal. In contrast, Appellants’ specification gives an example of a useful heterologous promoter in this regard, the cytomegalovirus promoter.

Furthermore, there is no disclose in Henderson or Little pertaining to detection of adenovirus ADP by western blot or cell spreading assay.

For the foregoing reasons, it is evident that the Action fails to make out a *prima facie* anticipation of the ADP overexpression claims. (For the sake of completeness, Applicants also incorporate by reference here the comments set forth below with respect to the remaining claims alleged to be anticipated).

**c)     *The Present Inventors Conceived of Vectors that Overexpress ADP, and their Use in Cancer Therapy, Before the Henderson/Little Priority Date, and Were Diligent in Reducing to Practice***

To demonstrate prior conception and diligence to reduction to practice, Appellants have attached and will rely on two separate declarations of the inventors now on file, including the declaration filed with the response dated 1/6/03 (“Wold I”; Exhibit 4) together with the



separately bound exhibits A-L to the Wold I declaration, and the supplemental declaration filed with the response dated September 3, 2003 (“Wold II”; Exhibit 5). It is noted that the Wold II declaration incorporates Wold I in its entirety. See para. 1 of Wold II. The two art references relied upon by the Examiner, Little and Henderson, have a priority date of no earlier than March 3, 1997.

**(1) The Research Proposal, Exhibit B to Wold I**

We would first direct the Board’s attention to Exhibit B of Wold I. This document is a research proposal dated well prior to 3/3/97, and describes the present inventors’ goal of preparing adenovirus vectors that overexpress the ADP gene (referred to here as the E3-11.6K gene). In section B of the proposal, the inventors describe the preparation of adenovirus mutants that express the ADP gene, and describe the function of the ADP gene to promote cell death. The inventors note that these earlier studies, using ADP+ and ADP- adenovectors, demonstrated that the ADP gene indeed functioned to promote cell death in infected cells. Based on these earlier studies, the inventors concluded that “[s]ince the 11.6K protein can promote the cell death of adenovirus-infected, it has the potential use as a therapeutic agent to kill cells, *e.g.*, malignant cell, in humans.” Section B, page 3. Questions are then posed as to how the ADP gene might be applied in human therapy – questions that are addressed in remaining portions of the exhibit.

Turning to page 6 of the Exhibit B research proposal, it is stated that one way to potentially achieve this goal is to construct a vector deleted in the E1A, E1B and E3 regions, wherein the ADP gene is reinserted in an expression cassette driven by the CMV promoter. Page 6, second full paragraph. Then, in section C.3., beginning on page 7, it is alternatively proposed to test the ability of an ADP expressing vector to overexpress the ADP gene (*i.e.*, the 11.6K gene) during early stages of infection by constructing a vector into which the “11.6K gene will be built in” yet which lacks all other E3 region genes and which contains all other adenovirus

genes. Such vectors are, in essence, the principal exemplary embodiments of the present invention.

Beginning on page 8, section D., the proposal goes on to suggest the preparation of vectors that “optimize expression” [viz, “overexpress”] of the ADP gene, and mention the possibility of preparing “nondefective” vectors (that is, replication competent vectors).

On pages 9-10 of the proposal, the inventors indicate that they will test the various constructs in animal models to assess their potential efficacy in treating cancer.

## **(2) Construction and Testing of KD1 Prior to 3/3/97**

The Wold I declaration provides additional evidence of conception prior to 3/3/97. In paragraph 5 of Wold I, the inventors detail the following studies conducted prior to 3/3/97:

- the observation that a mutation in the ADP resulted in the slower development of infection as compared to vectors having wild-type ADP;
- the observation that when target cancer vectors prepared with portions of the E3 region removed + reinsertion of the ADP gene resulted in larger plaques, and developed plaques faster, than wild-type (see third bullet point of paragraph 5); and
- gel electrophoresis and immunofluorescence studies demonstrating that E3-deleted, ADP expressing vectors produced higher levels of ADP than wild-type (fourth bullet point of paragraph 5).

Then, in paragraph 6 of Wold I, the inventors provide details on the construction of an ADP overexpressing vector prior to 3/3/97. This is the KD1 vector described in the specification (see, *e.g.*, page 22, Table 1) and which comprises the removal of E3 genes and reinsertion of the ADP gene. In particular, paragraph 6 and the supporting documentation demonstrates that KD1 was

actually constructed prior to 3/3/97 and the structure of the E3 region and ADP gene of KD1 was as expected (see, *e.g.*, page 4).

Thus, prior to the filing date of Henderson/Little, the present inventors had conceived of the idea of preparing vectors with E3 deletions + reinsertion of the ADP gene for the purpose of testing overexpression of ADP and for the purpose of developing a cancer therapeutic. Further, they demonstrated that such constructs overexpressed ADP as compared to wild-type and actually prepared the KD1 vector prior to that time. All that remained was testing of the KD1 vector to confirm that it did overexpress ADP (although this had already been shown by them with another such vector) and to show its ability to kill cancer cells in animals. Both of these objectives were diligently attained by the inventors in very short order.

### **(3) Confirmation that KD1 Overexpressed ADP**

Turning now to paragraph 7 of Wold I, the inventors detail studies on KD1 that were conducted in March, April and May of 1997, and present relevant studies that were carried out on KD1 and the KD vectors on virtually every day during those months. In particular, it is shown that at least as early as 5/9/97 to 5/23/97, the inventors conducted plaque assays on KD1 which demonstrated that KD-1 overexpressed ADP. See entries on page 8 of Wold I under the headings "5/9/97 – 5/23/97" and "5/13/97 – 6/2/97".

### **(4) Successful Animal Testing**

The Wold II declaration demonstrates the successful testing of KD1 in an animal having cancer. The inventors there show that on July 7, 1997, they sent KD1, dl1101/1107, dl309, and A549 cells to Dr. Jeffrey A. Whitsett at the Children's Hospital Medical Center, Division of Pulmonary Biology, Cincinnati, OH. A copy of the cover letter sent to Dr. Whitsett is attached to Wold II as Exhibit M. They state that Dr. Whitsett had agreed to test these vectors on their behalf in the A549 nude mouse model, in which A549 human lung carcinoma cells are used to

establish tumors in nude mice following by injection of the various vectors to determine their anticancer efficacy. On September 16, 1997, the inventors received a report from Dr. Whitsett's colleague, Lee Zhang, indicating that "10<sup>9</sup> pfu of each of the viruses were injected into each established A549 tumor. 4 out of 6 tumors injected with KD1 showed slowed tumor growth while 2 out of 2 tumors injected with dl309 and 4 out of 4 injected with dl 1101/1107 continued to grow." A copy of this fax is attached to Wold II as Exhibit N.

From the foregoing it is respectfully submitted that conception plus diligence to a reduction to practice has been shown.

In the Final Action, the Examiner raises various points in rebuttal, which Appellants would like to address:

First, in the paragraph bridging pages 10-11, the Action states that Exhibit B to Wold I is merely a research proposal, that it teaches away from using vectors that are "replication competent" in neoplastic cells and that there was no nexus between the Exhibit B proposal and KD1. In response, it is submitted that whether or not the exhibit is a research proposal is irrelevant where, as here, it denotes the inventor's goal and provides a road map to how they planned to achieve that goal. Further, the proposal does suggest the construction of "nondefective" replication competent vectors at D.1. on page 8. Lastly, while the proposal does not mention KD1 by name, it certainly contemplates the principal attribute of KD1 at issue here – that it comprises an E3-deletion with reinsertion of the ADP gene for "optimization" (*i.e.*, overexpression) of ADP during early stages of infection (as contrasted with late stages).

The Action further states that it was not known prior to 3/3/97 whether KD1 overexpressed ADP. This argument by the Examiner is not particularly relevant in that it overlooks the fact that the declaration DOES demonstrate that the inventors had prepared

OTHER vectors with a deleted E3 region and inserted ADP gene, very similar to KD1, and demonstrated that these vectors overexpressed ADP. See bullets 3 and 4 of paragraph 5 of Wold I. Thus, it is clear from the evidence that the inventors had constructed other similar vectors prior to 3/3/97 that were shown to overexpress ADP, and it is evident that it was their intention in preparing KD1 that it have this property as well. It was indeed confirmed to have this desirable property within two months of 3/3/97 after diligent work toward this goal.

The Final Action, at page 11, also takes the position that the declarations do not demonstrate conception of the claimed invention as a whole. This argument is disagreed with on at least two bases: one, an antedating declaration under Rule 131 need only show so much as the prior art shows, which has been done, and, two, there is no requirement that a Rule 131 declaration demonstrate a reduction to practice of an entire genus.

First, the law is clear that a Rule 131 declaration need only show so much as the prior art discloses. See, *e.g.*, *In re Stempel*, 113 U.S.P.Q. 77 (CCPA 1957). Thus, it is submitted that the present Applicants carried out in the United States studies that demonstrate the reduction to practice of an ADP-overexpressing adenovirus and successful demonstration of tumor growth suppression prior to the March, 1998, effective filing date of the CN751 description in Little/Henderson. Under the doctrine of *In re Stempel*, it is submitted that the Little/Henderson references have been antedated inasmuch as the Rule 131 showing is at least commensurate in scope with that found in the March 1997 and March 1998 respective filing dates of the Henderson/Little patents.

Furthermore, the law is also clear that Rule 131 declarations are not required to set forth a reduction to practice across the claimed genus. For example, in *In re Hostettler*, 148 U.S.P.Q. 514 (CCPA 1966), the court was adamant in reversing the PTO on this very point:

Rule 131 requires applicant to make oath to facts showing a completion “of the invention.” That requirement does not mean affiant must show a reduction to practice of every embodiment of the invention. Nor is that requirement coextensive with the amount of disclosure necessary to support a claim under 35 USC 112.

148 U.S.P.Q. at 516.

Furthermore, the fact that Appellants’ reduction to practice was demonstrated with a vector having some differences from CN751 in no way undermines Appellants’ claim to priority. The fact is, if there are differences, the differences are not relevant to the fundamental issue of who first demonstrated reduction to practice of the central concept of expressing or overexpressing ADP from a recombinant adenovirus for the purposes of cancer therapy. That showing has clearly been made by the present Applicants. Furthermore, the case law again strongly supports our position. For example, *In re Clarke*, 148 U.S.P.Q. 665 (CCPA 1966), presented the situation where the Rule 131 affidavit described embodiments that were similar to but distinct from the disclosure contained in the reference at issue. In reversing the Board and the examiner, the court observed that:

We believe that Stempel ... is not limited to the fact situations where the inventor can show priority as to the identical compound described in the reference. It seems that in an appropriate case as applicant should not be prevented from obtaining a patent to an invention where a compound described in a reference would have been obvious to one of ordinary skill in that art in view of what the affiant proves was completed with respect to the invention prior to the effective date of the reference.

148 U.S.P.Q. at 670. Here, the fact that Applicants’ earlier-reduced-to-practice KD1 included the 01/07 E1 mutation in addition to the upregulating ADP/E3 mutations should thus in no way detract from the ability of KD1 to effectively antedate a reference teaching the ADP/E3 mutations without the 01/07 mutations. Stated another way, KD1 would appear to anticipate or

at least obviate CN751, at least to the extent that both are principally concerned with vectors that express ADP as the operative lytic agent.

Therefore, in view of the above, the “overexpress ADP” claims are not anticipated by Henderson or Little.

**2.      *The Structural Claims – Claims 60, 61, 68, 69, 72-75, 97-99, 107***

The Action rejects structural claims 60, 61, 68, 69, 72-75, 97-99, and 107 as anticipated by Henderson and Little, noting merely that these claims do not require overexpression of ADP.

Henderson and Little are not available as prior art because Appellants have demonstrated prior conception and diligence to reduction to practice in advance of the priority dates of Henderson and Little. The foregoing evidence of conception and reduction to practice, incorporated herein by reference, fully applies to claim 60 and claims depending therefrom.

As noted above, Appellants have attached and relied on two separate declarations of the inventors now on file, including the declaration filed with the response dated January 6, 2003 (“Wold I”; Exhibit 4) together with the separately bound exhibits A-L to the Wold I declaration, and the supplemental declaration filed with the response dated September 3, 2003 (“Wold II”; Exhibit 5). As noted above, the Wold II declaration incorporates Wold I in its entirety. See para. 1 of Wold II. The two art references relied upon by the Examiner, Little and Henderson, have a priority date of no earlier than March 3, 1997.

As discussed above, prior to the filing date of Henderson and Little, the present inventors had conceived of the idea of preparing vectors with E3 deletions plus reinsertion of the ADP gene for the purpose of testing overexpression of ADP and for the purpose of developing a cancer therapeutic. Further, they demonstrated that such constructs overexpressed ADP as compared to wild-type and actually prepared the KD1 vector prior to that time. All that remained was testing of the KD1 vector to confirm that it did overexpress ADP (although this

had already been shown by them with another such vector) and to show its ability to kill cancer cells in animals. Both of these objectives were diligently attained by the inventors in very short order.

As noted above, the structural claims do not require overexpression of ADP. Rather than requiring “overexpression” of ADP, these claims require an adenovirus vector having certain structural features. For example, according to independent claim 60, the adenovirus vector must be replication-competent in neoplastic cells, and must express an ADP, wherein: (1) the ADP is expressed from an ADP coding sequence positioned under the control of a promoter other than the endogenous promoters for ADP; (2) the adenovirus vector comprises a deletion in the E3 region that removes a splice site for an E3 mRNA; (3) the ADP is expressed from an ADP coding sequence flanked by a pre-mRNA splicing and cleavage/polyadenylation signal other than the pre-mRNA splicing and cleavage/polyadenylation signal normally associated with the ADP gene; and/or (4) the ADP is expressed from an ADP coding sequence that is positioned downstream of the coding sequence for another adenovirus mRNA, together with a sequence on the 5’ side of the ADP coding sequence that allows for internal initiation of translation of ADP. Thus, there is *no* requirement that the adenovirus vectors required for these claims must overexpress ADP.

Although the present inventors have provided evidence that their vectors overexpressed ADP, they were not required to do so since the structural claims do not require a vector that overexpresses ADP. Furthermore, the fact that the structural claims do not require overexpression renders irrelevant the Examiner’s argument that KD1 was not shown to overexpress ADP prior to Mar. 3, 1997.



In view of the above, Henderson and Little are not available as prior art. Accordingly, the Board is requested to overturn the Examiner's position regarding anticipation.

**D. Rejection of claims 13, 20-22, 60, and 64-66 under 35 U.S.C. §103(a) over Henderson *et al.* ("Henderson"; Exhibit 1) or Little *et al.* ("Little"; Exhibit 2) as applied to claims 10-13, 32-44, 60, 61, 68, 69, 72-75, 97-99, and 101-108, and further in view of Freytag (Exhibit 3)**

The Action next rejects claims 13, 20-22, 60 and 64-66 as obvious over Henderson and Little in light of Freytag. Henderson and Little are discussed above. Freytag is alleged to teach combination therapy using adenoviruses and chemotherapy and/or radiation. Appellants respectfully traverse.

**1. *Henderson and Little are not Available as Prior Art, and Freytag Alone Fails to Teach or Suggest the Claimed Invention***

For the reasons discussed above, which are specifically incorporated into this section, Henderson and Little are not available as prior art. As set forth above, Henderson and Little are not available as prior art because Appellants have demonstrated prior conception and diligence to reduction to practice in advance of the priority dates of Henderson and Little. This is true for both the "overexpress ADP" claims at issue in this rejection (*i.e.*, claims 13, and 20-22) and the structural claims at issue in this rejection (*i.e.*, claims 60 and 64-66).

In order to establish a *prima facie* case of obviousness, three basic criteria must be met: (1) the prior art reference (or references when combined) must teach or suggest all the claim limitations; (2) there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings; (3) there must be a reasonable expectation of success. *Manual of Patent Examining Procedure* § 2142. See also *In re Vaeck*, 947 F.2d 488, 20 U.S.P.Q. 2d 1438 (Fed Cir. 1991) (emphasizing that the teaching or suggestion to make the

claimed combination and the reasonable expectation of success must be both found in the prior art, and not based on applicant's disclosure). It is important to note that all three elements must be shown to establish a *prima facie* case of obviousness. Thus, if one element is missing, a *prima facie* case of obviousness does not exist.

Freytag alone fail to teach or suggest the claimed invention. Indeed, Freytag has no bearing on the claimed invention. Freytag pertains to a replication competent, E1B-attenuated so-called suicide gene adenovirus vector comprising the cytosine deaminase (CD)/herpes simplex virus type-1 thymidine kinase gene. There is no mention of an ADP gene, vectors employing the ADP gene, or upregulated ADP in Freytag. This suicide gene construct is specially constructed to be used with radiation and chemotherapy. There is no motivation to employ radiation except in connection with vectors that bear the special suicide CD/HSV-1 TK gene construct. Accordingly, there is no basis in this reference to arrive at the invention of claims 13, 20-22, 60 or 64-66. Thus, no *prima facie* rejection has been established.

**2. *Even if Henderson and Little were Available as Prior Art, there is no Prima Facie Case of Obviousness***

As set forth above, Appellants have demonstrated that Henderson and Little are not available as prior art. Even if these references were available as prior art (and Appellants herein make no such admission), there would still be no *prima facie* case of obviousness.

With respect to independent claims 13 and 60, Freytag adds nothing to the teachings of Henderson and Little that is relevant to the subject matter of these claims. As to claim 13, Freytag fails to teach or suggest an adenovirus vector that overexpresses ADP. As to claim 60, Freytag fails to teach or suggest the structural limitations set forth in claim 60.

With respect to the dependent claims, Freytag merely discloses a so-called suicide gene adenovirus vector comprising the CD/HSV-1 TK gene. This suicide gene construct is specially

constructed to be used with radiation and chemotherapy. As noted above, there is no mention of an ADP gene or vectors employing the ADP gene or upregulated expression of ADP, and thus no motivation to employ radiation except in connection with vectors that bear this special suicide CD/HSV-1 TK gene construct. In addition, there is no motivation to employ chemotherapy except in connection with vectors that bear the special suicide CD/HSV-1 TK gene construct. Accordingly, there is no basis for combining this reference with the ADP teachings of Henderson and Little to arrive at the invention of claims 13, 20-22, 60, or 64-66. Thus, no *prima facie* case of obviousness has been established.

Accordingly, for each of the foregoing reasons, the Board is requested to overturn the Examiner's position regarding obviousness of the claimed invention.

**E. Double Patenting of Claims 10-13, 32-44, 60, 61, 72-75, and 97-108**

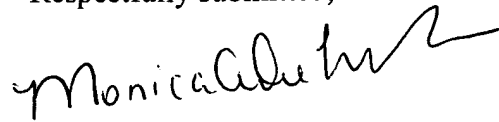
Claims 10-13, 32-44, 60, 61, 72-75 and 97-108 are rejected under the judicially-created doctrine of obviousness-type double patenting as being unpatentable over claims 1-11 of U.S. Patent 6,627,190. Without conceding that the claims at issue are not patentably distinct from the claims of the '190 patent, Applicants will remove the issue of double patenting for these claims by filing a terminal disclaimer. The terminal disclaimer has been filed concurrently with submission of this appeal brief, and a copy is attached as Appendix 3.

**VIII. CONCLUSION**

Appellants have provided arguments that overcome the pending rejections. Appellants respectfully submit that the Office Action's conclusions that the claims should be rejected are unwarranted. It is therefore requested that the Board overturn the Action's rejections.

Please date stamp and return the enclosed postcard to evidence receipt of this document.

Respectfully submitted,

A handwritten signature in cursive script, appearing to read "Monica A. De La Paz", with a long, sweeping flourish extending to the right.

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Date: November 9, 2004

## APPENDIX 1

### CLAIMS APPENDIX

11. The method of claim 13 wherein the adenovirus death protein comprises SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, or SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11 or SEQ ID NO:12.

12. The method of claim 13, wherein the adenovirus vector comprises a recombinant adenovirus lacking expression of at least one E3 protein selected from the group consisting of: gp19K; RID $\alpha$ ; RID $\beta$  and 14.7K.

13. A method for treating cancer in an animal having a tumor comprising administering to the tumor an adenovirus vector wherein said adenovirus vector is replication-competent in neoplastic cells and overexpresses an adenovirus death protein (ADP), wherein overexpression is defined as overexpression relative to *dI309*.

14. The method of claim 13, further comprising the step of passively immunizing the animal against the adenovirus vector.

15. The method of claim 14, wherein the adenovirus vector comprises SEQ ID NO:1 or SEQ ID NO:2.

20. The method of claim 13, further comprising treating the tumor with radiation.

21. The method of claim 20 comprising administering more than one distinct type of recombinant adenovirus to the tumor and treating the tumor with radiation, wherein at least one recombinant adenovirus is replication-defective.

22. The method of claim 13, further comprising treating the tumor with chemotherapy.

24. The method of claim 13, further comprising administering to the tumor one or more replication-defective adenoviruses, wherein each replication-defective adenovirus expresses an anti-cancer gene product, and wherein the adenovirus vector facilitates the spread of the replication-defective adenovirus in the tumor.

32. The method of claim 13, wherein overexpression relative to *dl309* is detectable by western blot, cell lysis, virus release or by a cell spreading assay.

33. The method of claim 13, wherein the adenovirus vector lacks expression of at least one E3 protein selected from the group consisting of gp19K, RID $\alpha$ , RID $\beta$  and 14.7K.

34. The method of claim 33, wherein the adenovirus vector lacks expression of the gp19K protein.

35. The method of claim 33, wherein the adenovirus vector lacks expression of the RID $\alpha$  protein.

36. The method of claim claim 33, wherein the adenovirus vector lacks expression of the RID $\beta$  protein.

37. The method of claim 33, wherein the adenovirus vector lacks expression of the 14.7K protein.

38. The method of claim 33, wherein the adenovirus vector lacks expression of the gp19K, RID $\alpha$ , RID $\beta$  and 14.7K proteins.

39. The method of claim 28, wherein the adenovirus vector comprises a deletion in the E3 region that removes a splice site for any of the E3 mRNAs.

40. The method of claim 13, wherein the adenovirus vector comprises at least one deletion in the E3 region, wherein the at least one deletion comprises a sequence that encodes at

least one E3 protein, wherein the protein is selected from the group consisting of gp19K, RID $\alpha$ , RID $\beta$ , and 14.7K.

41. The method of claim 40, wherein the at least one deletion comprises a sequence that encodes the gp19K, RID $\alpha$ , RID $\beta$  and 14.7K proteins.

42. The method of claim 41, wherein the at least one deletion further comprises a sequence that encodes the 6.7K protein.

43. The method of claim 41, wherein the at least one deletion further comprises a sequence that encodes the 12.5K protein.

44. The method of claim 41, wherein the at least one deletion further comprises a sequence that encodes the 6.7K and 12.5K proteins.

60. A method for treating cancer in an animal having a tumor, the method comprising administering to the tumor an adenovirus vector that is replication-competent in neoplastic cells and expresses an adenovirus death protein (ADP), wherein:

- a) the ADP is expressed from an ADP coding sequence positioned under the control of a promoter other than the endogenous promoters for ADP;
- b) the adenovirus vector comprises a deletion in the E3 region that removes a splice site for an E3 mRNA;
- c) the ADP is expressed from an ADP coding sequence flanked by a pre-mRNA splicing and cleavage/polyadenylation signal other than the pre-mRNA splicing and cleavage/polyadenylation signal normally associated with the ADP gene, and/or
- d) the ADP is expressed from an ADP coding sequence that is positioned downstream of the coding sequence for another adenovirus mRNA, together with a sequence on the 5' side of the ADP coding sequence that allows for internal initiation of translation of ADP.

61. The method of claim 60 wherein the ADP comprises the sequence of SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11 or SEQ ID NO:12.

62. The method of claim 60, further comprising the step of passively immunizing the animal against the adenovirus vector.

63. The method of claim 62, wherein the adenovirus vector comprises SEQ ID NO:1 or SEQ ID NO:2.

64. The method of claim 60, further comprising treating the tumor with radiation.

65. The method of claim 64 comprising administering more than one distinct type of recombinant adenovirus to the tumor and treating the tumor with radiation, wherein at least one recombinant adenovirus is replication-defective.

66. The method of claim 60, further comprising treating the tumor with chemotherapy.

67. The method of claim 60, further comprising administering to the tumor one or more replication-defective adenoviruses, wherein each replication-defective adenovirus expresses an anti-cancer gene product, and wherein the adenovirus vector facilitates the spread of adenoviruses in the tumor.

68. The method of claim 60, wherein the ADP is expressed from an ADP coding sequence positioned under the control of promoter other than the endogenous promoters for ADP.

69. The method of claim 68, wherein the ADP coding sequence is positioned under the control of a promoter that is exogenous to adenovirus.



70. The method of claim 60, wherein the ADP coding sequence is positioned behind a coding sequence for another adenovirus mRNA together with a sequence on the 5' side of the ADP coding sequence that allows for internal initiation of translation of ADP.

71. The method of claim 70, wherein the sequence on the 5' side of the ADP coding sequence that allows for internal initiation of translation of ADP is an Ad tripartite leader or a viral internal ribosome initiation sequence.

72. The method of claim 60, wherein the adenovirus vector comprises a deletion in the E3 region that removes a splice site for an E3 mRNA.

73. The method of claim 72, wherein the adenovirus vector lacks expression of at least one E3 protein selected from the group consisting of gp19K, RID $\alpha$ , RID $\beta$ , and 14.7K.

74. The method of claim 73, wherein the adenovirus vector lacks expression of each of gp19K, RID $\alpha$ , RID $\beta$ , and 14.7K.

75. The method of claim 74, wherein the adenovirus additionally lacks expression of the E3 6.7K and 12.5K proteins.

97. The method of claim 60, wherein the adenovirus vector is an Ad1, Ad2, Ad5 or Ad6 vector.

98. The method of claim 60, wherein the adenovirus vector is administered to the tumor by injection of vector intravenously or intrathecally.

99. The method of claim 60, wherein the adenovirus vector is administered to the tumor by direct injection of the tumor.

100. The method of claim 60, wherein the animal is passively immunized against the recombinant adenovirus.

101. A method for treating cancer in an animal having a tumor, the method comprising administering to the tumor an adenovirus vector wherein said adenovirus vector is replication-competent in neoplastic cells and overexpresses an adenovirus death protein (ADP).

102. A method for treating cancer in an animal having a tumor, the method comprising administering to the tumor an adenovirus vector wherein said adenovirus vector is replication-competent in neoplastic cells and overexpresses an adenovirus death protein (ADP), wherein overexpression is effected by one of more of the following modifications:

- a) the ADP is expressed from an ADP coding sequence positioned under the control of a promoter other than the endogenous promoters for ADP;
- b) the adenovirus vector comprises a deletion in the E3 region that removes a splice site for an E3 mRNA;
- c) the ADP is expressed from an ADP coding sequence flanked by a pre-mRNA splicing and cleavage/polyadenylation signal other than the pre-mRNA splicing and cleavage/polyadenylation signal normally associated with the ADP gene, and/or
- d) the ADP is expressed from an ADP coding sequence that is positioned downstream of the coding sequence for another adenovirus mRNA, together with a sequence on the 5' side of the ADP coding sequence that allows for internal initiation of translation of ADP.

103. The method of claim 32, wherein the overexpression relative to a control virus is detectable by western blot

104. The method of claim 32, wherein the overexpression relative to a control virus is detectable by cell lysis

105. The method of claim 32, wherein the overexpression relative to a control virus is detectable by virus release

106. The method of claim 32, wherein the overexpression relative to a control virus is detectable by a cell spreading assay.

107. The method of claim 60, wherein the animal is a human.

108. The method of claim 13, wherein the animal is a human.

## **APPENDIX 2**

### **EVIDENCE APPENDIX**

1. Henderson *et al.* (U.S. Patent 6,197,293): Cited in Office Action dated March 15, 2001.
2. Little *et al.* (U.S. Patent 6,254,862): Cited in Office Action dated August 24, 2001.
3. Freytag *et al.* (Hum. Gene Ther. 9:1323-1333, 1998): Cited in Office Action dated March 15, 2001.
4. Wold I Declaration (with Exhibits A-L): Filed on January 3, 2002, in response to the Office Action dated August 24, 2001.
5. Wold II Declaration (with Exhibits M-N): Filed on December 3, 2003, in response to the Office Action dated September 3, 2003.
6. Terminal Disclaimer: Submitted with Supplemental Appeal Brief dated Nov. 3, 2004.

## A Novel Three-Pronged Approach to Kill Cancer Cells Selectively: Concomitant Viral, Double Suicide Gene, and Radiotherapy

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### ABSTRACT

Two obstacles limiting the efficacy of nearly all cancer gene therapy trials are low gene transduction efficiencies and the lack of tumor specificity. Recently, a replication-competent, E1B-attenuated adenovirus (ONYX-015) was developed that could overcome these limitations, because it was capable of efficiently and selectively destroying tumor cells lacking functional p53. In an attempt to improve both the efficacy and safety of this approach, we constructed a similar adenovirus (FGR) containing a cytosine deaminase (CD)/herpes simplex virus type-1 thymidine kinase (HSV-1 TK) fusion gene, thereby allowing for the utilization of double-suicide gene therapy, which has previously been demonstrated to produce significant antitumor effects and potentiate the therapeutic effects of radiation. The FGR virus exhibited the same tumor cell specificity and replication kinetics as the ONYX-015 virus *in vitro*. Importantly, both the CD/5-FC and HSV-1 TK/GCV suicide gene systems markedly enhanced the tumor cell-specific cytopathic effect of the virus, and, as expected, sensitized tumor cells to radiation. By contrast, neither the FGR virus nor either suicide gene system showed significant toxicity to normal human cells. Both suicide gene systems could be used to suppress viral replication effectively, thereby providing a means to control viral spread. The results support the thesis that the three-pronged approach of viral therapy, suicide gene therapy, and radiotherapy may represent a powerful and safe means of selectively destroying tumor cells *in vivo*.

### OVERVIEW SUMMARY

In this study, we examine the hypothesis that expression of a CD/HSV-1 TK fusion gene may improve both the efficacy and safety of cancer therapies involving replication-competent, E1B-attenuated adenoviruses. Both the CD/5-FC and HSV-1 TK/GCV suicide gene systems markedly enhanced the tumor cell-specific cytopathic effects of such viruses and sensitized tumor cells to radiation. Neither the virus nor the suicide gene systems showed significant toxicity to normal human cells. Both suicide gene systems were effective at suppressing viral replication, thereby providing a safety mechanism to halt virus spread. We propose that the three-pronged approach of viral therapy, suicide gene therapy, and radiotherapy may ultimately prove to be a safe and effective means of selectively destroying tumor cells *in vivo*.

### INTRODUCTION

**D**ESPITE ADVANCES IN BOTH diagnosis and therapy, the annual number of cancer-related deaths has not decreased during the past 60 years. Although conventional cancer therapies (surgery, radiotherapy, chemotherapy) produce a high rate of cure for patients with early-stage disease, many cancers recur and the majority of patients with advanced cancer eventually succumb to the disease. The limitations of conventional cancer therapies do not derive from their inability to ablate tumor, but rather from limits on their ability to do so without excessively damaging the patient. It is this consideration that constrains the extent of surgical resection, the dose of radiation and volume to be irradiated, and the dose and combination of chemotherapeutic drugs. Improving the effectiveness of a treatment is of no clinical value if there is no significant increase

in the differential response between tumor and normal tissue (i.e., the therapeutic index).

As a means of increasing the therapeutic index, several new approaches involving gene therapy are under development. One of these approaches, suicide gene therapy, involves the transfer and expression of nonmammalian genes encoding enzymes that convert nontoxic prodrugs into toxic antimetabolites. Two suicide genes currently being tested in clinical trials are the *Escherichia coli* cytosine deaminase (CD) and herpes simplex type-1 thymidine kinase (HSV-1 TK) genes, which confer sensitivity to 5-fluorocytosine (5-FC) and ganciclovir (GCV), respectively (DeClercq, 1984; Calabresi and Chabner, 1990). The rationale behind the suicide gene therapy approach is that, following targeted transfer of these genes to the tumor site, only the tumor and neighboring cells (via bystander effects) will be rendered sensitive to their cytotoxic action. Thus, with this approach, systemic toxicity commonly associated with, and a major limitation of, conventional chemotherapy [e.g. 5-fluorouracil (5-FU)] is avoided. We (Rogulski *et al.*, 1997b) and others (Moolten and Wells, 1990; Moolten, 1994; Ezzeddine *et al.*, 1991; Culver *et al.*, 1992; Mullen *et al.*, 1992, 1994; Huber *et al.*, 1993, 1994; Takamiya *et al.*, 1993; Chen *et al.*, 1994; Hirschowitz *et al.*, 1995; Richards *et al.*, 1995; Cool *et al.*, 1996; Dong *et al.*, 1996; Ohwada *et al.*, 1996) have demonstrated the efficacy and safety of suicide gene therapy in animal models. Using retrovirally transduced cells, we have achieved 100% regression of very large intramuscular tumors (3 cm<sup>3</sup>) using the CD/5-FC and HSV-1 TK/GCV systems simultaneously with no side effects (Rogulski *et al.*, 1997b). It is noteworthy that the size of these experimental tumors is within the range of those observed in human cancer patients. These results give us reason to believe that the suicide gene therapy approach could be efficacious against some human tumors providing that the genes can be delivered efficiently to the tumor site. Unfortunately, a current limitation of nearly all cancer gene therapy approaches is that the efficiency at which therapeutic genes can be delivered *in vivo* is too low to make them efficacious against most human cancers.

A novel approach with much potential is the use of replication-competent, mutant adenoviruses that preferentially replicate in tumor cells (Bischoff *et al.*, 1996; Heise *et al.*, 1997). The prototype ONYX-015 is a replication-competent mutant adenovirus that fails to express the 55-kD E1B protein, which binds to and inactivates the cellular tumor suppressor p53 (Yew and Burke, 1992). As a result, this virus replicates in, and preferentially kills, cells lacking functional p53. The strength of this approach is that it exploits a major biological difference between normal and tumor cells, which should result in an increase in the therapeutic index. Moreover, the replication capacity of the virus should dramatically increase the efficiency of its antitumor effect. However, despite its noted strengths, this approach has limitations. For example, there is no reason to think that E1B-attenuated viruses, such as ONYX-015, could selectively destroy tumors cells containing functional p53. This point may be important, because many human tumors exhibit significant intratumoral heterogeneity with respect to p53 status (Mirchandani *et al.*, 1995; Yang *et al.*, 1996). Because adenoviruses are immunogenic, this approach will elicit an immunological response that may not only attenuate its antitumor effects, but may also preclude repeated doses. Finally, the ability of the virus to replicate, albeit ineffi-

ciently, in normal human cells, may raise concerns regarding the safety of this approach. Thus, it would be desirable to combine this approach with one that could (i) expand the spectrum and tumor cell types that it would be effective against, (ii) increase its therapeutic efficacy per dose, and (iii) control the replication and spread of the virus.

With these goals in mind, we developed a replication-competent, E1B-attenuated adenovirus similar to ONYX-015 that contains a CD/HSV-1 TK fusion gene. We demonstrate here that this virus exhibits all the advantages of the ONYX-015 virus, but in addition, is improved significantly in both efficacy and safety.

## MATERIALS AND METHODS

### Plasmids, viruses, antibodies, and cell lines

All plasmids containing adenoviral sequences used in the construction of a replication-competent, E1B-attenuated adenovirus containing a CD/HSV-1 TK fusion gene (FGR) were obtained from Microbix (Toronto, Canada). Construction and characterization of the CD/HSV-1 TK fusion gene has been previously described (Rogulski *et al.*, 1997a). Wild-type adenovirus type 5 and ONYX-015 were obtained from ONYX Pharmaceuticals (Richmond, CA). U251, DU145, PC-3, HT29, and MRC-5 cells were obtained from the American Type Culture Collection (ATCC). 293 cells were obtained from Microbix. Antibodies to the adenovirus type 2 E1A (sc-430) and 55-kD E1B (DP08) proteins were obtained from Calbiochem (LaJolla, CA).

### Construction of FGR and production of adenoviruses

The wild-type E1 region from bases 343 to 2,270 was generated by the polymerase chain reaction (PCR) using pXC1 (Microbix) as template. The resulting 1,928-bp fragment contained *Bam* HI restriction sites at each end, a *Bgl* II restriction site 5' to the 3' *Bam* HI site, and two mutations at bases 2,253 (C → T) and 2,262 (G → T) generating premature translation stop codons in the 55-kD E1B reading frame at codons 79 and 82, respectively. The PCR fragment was digested with *Bam* HI and cloned into the 6.4kb fragment of pCA14 (Microbix) generating pCA14-E1aE1b. The CD/HSV-1 TK fusion gene was removed from pWZLneoCDglyTK (Rogulski *et al.*, 1997a) by a partial *Bam* HI-*Eco* RI digestion and cloned between the *Bam* HI and *Eco* RI sites of pCA14 generating pCA14-CDglyTK. The entire expression cassette, containing the cytomegalovirus (CMV) promoter, CDglyTK fusion gene and SV40 polyadenylation elements, we removed from pCA14-CDglyTK by *Bgl* II digestion. The 3.1-kb fragment was cloned into the sole *Bgl* II site of pCA14-E1aE1b generating pCA14-E1aE1b/CDglyTK. Both pCA14-E1aE1b/CDglyTK and pCA14-CDglyTK were linearized with *Pvu* I and co-transfected with *Cla* I-linearized pBHG10 (Microbix) into 293 cells to generate the FGR virus and its replication-defective counterpart (Ad5-CDglyTK). Isolated plaques were picked 10–14 days later and plaque-purified on 293 cells a second time. All viruses were propagated in 293 cells to generate crude viral supernatants (titers 20–20 × 10<sup>8</sup> pfu/ml) or CsCl gradient-purified virus (titers 5–50 × 10<sup>11</sup> pfu/ml). All viral preparations were assayed for their ability to

replicate in and lyse non-E1A-expressing cells, for genome integrity and stability by Southern blotting, and for expression of the CDglyTK fusion gene by Western blotting and immunofluorescence.

#### Western blotting

Cells ( $1 \times 10^6$ , 60-mm dish) were infected with the various crude viral supernatants at a multiplicity of infection (moi) of 10 in 0.2 ml of Dulbecco's modified Eagle's medium (DMEM). After 1 hr, fresh growth media was added. Eighteen hours post-infection, cells were lysed in Laemmli sample buffer. Samples (15  $\mu$ l) containing protein from  $3 \times 10^5$  cells were applied to a SDS-10% polyacrylamide gel and transferred to nitrocellulose using standard procedures. Blots were probed with a polyclonal antibody directed against the adenovirus type 2 E1A protein (sc-430). Antibody binding was visualized using standard chemiluminescence procedures.

#### Cytosine deaminase assays and immunofluorescence

Cells ( $1 \times 10^6$ , 60-mm dish) were harvested 48 hr post adenoviral infection. Cell extracts were prepared and cytosine deaminase (CD) assays performed as previously described (Rogulski *et al.*, 1997a). For each reaction, 50  $\mu$ g of protein was used. For CD immunofluorescence, tumors were excised, frozen, and sectioned. Samples were placed on glass slides, fixed in methanol, and washed thoroughly with phosphate-buffered saline (PBS). Samples were incubated with a polyclonal antibody to *E. coli* CD (provided by C. Richards, Glaxo-Wellcome), followed by a Texas Red-conjugated donkey anti-rabbit antibody. Samples were stained with DAPI and photographed using an Olympus fluorescent microscope.

#### Immunoprecipitation

For immunoprecipitation of the adenovirus 55-kD E1B protein, cell monolayers were incubated in 4 ml of DMEM containing 2% dialyzed fetal calf serum (FCS) and 100  $\mu$ Ci/ml [ $^{35}$ S]methionine/cysteine (ICN; TRANS $^{35}$ SLABEL; 1,439 Ci/mmol) for 2 hr beginning 16 hr post-infection. Cell monolayers were washed with PBS and lysed directly in 1 ml of RIPA buffer (9.1 mM NaH<sub>2</sub>PO<sub>4</sub>, 1.7 mM NaH<sub>2</sub>PO<sub>4</sub>, 150 mM NaCl, 1% Nonidet P-40, 0.5% Na deoxycholate, 0.1% SDS pH 7.4). Cell extracts were clarified by microcentrifugation, and preabsorbed with 1  $\mu$ g of normal rabbit serum and 20  $\mu$ l of Protein A/G PLUS Agarose (Santa Cruz Biotechnology Inc.). One microgram of the monoclonal anti-55-kD E1B antibody (DP08) was added to 0.5 ml of clarified cell extract and incubated on ice for 1 hr. Twenty microliters (20  $\mu$ l) of Protein A/G PLUS Agarose was added and the sample was incubated at 4°C for 1 hr with constant mixing. The immunoprecipitates were collected by microcentrifugation and washed four times with 1 ml of RIPA buffer followed by one wash with 1 ml of PBS. The final pellet was resuspended in 40  $\mu$ l of Laemmli sample buffer, heated at 100°C for 5 min, and the agarose was pelleted by microcentrifugation. Twenty microliters of the supernatant fractions were applied to an SDS-10% polyacrylamide gel and the dried gel were subjected to autoradiography.

#### Cytopathic effect (CPE)

To compare the viral cytopathic effects, cells ( $10^5$ /well, 24-well plate) were either mock-infected, or infected with increasing moi in 0.2–0.4 ml of DMEM. After 1 hr, the virus was removed and cells were incubated in DMEM containing 10% FCS (growth medium). Duplicate plates were fixed and stained with crystal violet either 5 or 9 days post-infection. For pro-drug sensitivity assays, cells ( $1 \times 10^6$ , 60-mm dish) were either mock-infected, or infected with virus at an moi of 10. After 1 hr, the cells were detached and replated ( $2 \times 10^4$  cells/well, 24-well plates) in either growth medium, or growth medium containing varying concentrations of prodrugs (GCV and/or 5-FC). The media was changed every 2 days. Duplicate plates were fixed and stained with crystal violet either 5 or 9 days later. To quantify the cytopathic effect, cells were replated in triplicate at low density ( $10^4$  and  $10^3$  cells/60-mm dish) immediately following infection. Colonies were fixed, stained with crystal violet, and counted 7–10 days later.

#### Assay for viral replication

Cells ( $1 \times 10^5$ /well, 24-well plate) were either mock-infected, or infected at an moi of 10 in 0.2 ml of DMEM. After 1 hr, the virus was removed and cells were incubated in DMEM containing 10% FCS. Cells were harvested every day thereafter by lysing them in 0.4 ml of 10 mM Tris-HCl pH 7.5, 10 mM NaCl, and 0.6% SDS. Genomic DNA was precipitated by the addition of 0.1 ml of 5 M NaCl and incubation at 4°C overnight. Following microcentrifugation, the supernatant fractions were extracted with phenol, CHCl<sub>3</sub>, and viral DNA was precipitated with ethanol. Samples were digested with *Hind* III and analyzed by Southern blotting using standard techniques. The blots were probed with [ $^{32}$ P]pBHG10 (Microbix), which detects all but the left-most *Hind* III fragment of all the viruses. To monitor prodrug inhibition of viral replication, cells ( $1 \times 10^6$ , 60-mm dish) were infected with FGR at a moi of 10 and immediately replated ( $2 \times 10^4$  cells/well, 24-well plate) in varying concentrations of prodrugs. Cells were harvested 48 hr post-infection and processed for Southern blot analysis as described above.

#### Radiosensitivity experiments

DU145 and U251 cells ( $2 \times 10^5$  cells/well, 6 well plate) were either mock-infected, or infected with FGR at a moi of 1. Cells were incubated in growth medium for 48 hr to allow for viral replication and expression of the CD/HSV-1 TK fusion gene. The medium was then replaced with normal growth medium or medium containing either 40  $\mu$ g/ml of 5-FC or 0.1  $\mu$ g/ml of GCV. After 24 hr, cells were exposed to a single dose of  $\gamma$ -irradiation (0–6 Gy). Cells were immediately replated in triplicate at low density ( $1 \times 10^4$  and  $1 \times 10^3$  cells/60-mm dish) in growth medium. Colonies were fixed, stained with crystal violet, and counted 7–10 days later. The sensitizer enhancement ratio (SER) represents the ratio between the D<sub>0</sub> of radiation alone and the D<sub>0</sub> for radiation plus virus and prodrugs. Cell survival data were analyzed using a nonlinear least square algorithm (Albright, 1987). Radiation cell survival curves were fit using the Single-Hit Multi-Target (SHMT) model.

## RESULTS

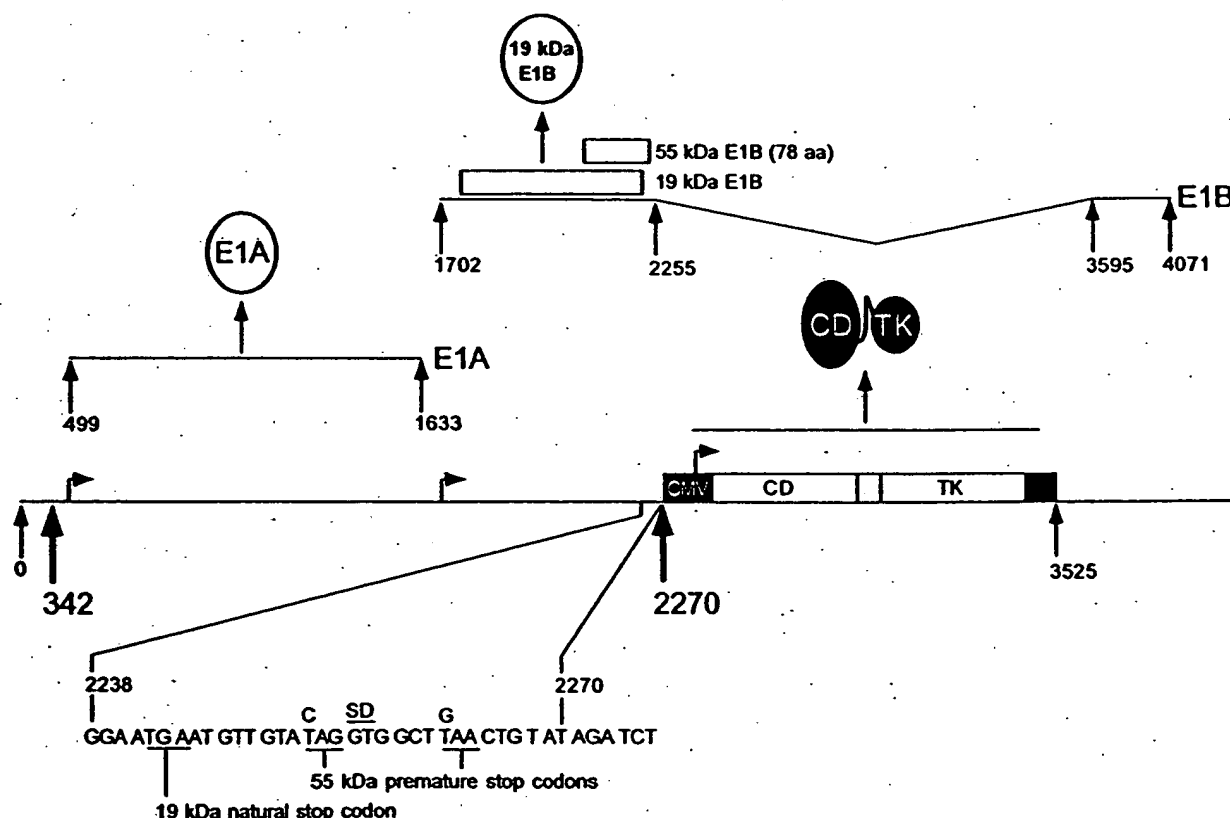
*Characterization of replication-competent, E1B-attenuated adenovirus containing CD/HSV-1 TK fusion gene*

A schematic representation of the E1 region of our replication-competent, E1B-attenuated adenovirus containing the CD/HSV-1 TK fusion gene (FGR) is shown in Fig. 1. Expression of the CDglyTK gene (Rogulski *et al.*, 1997a) is driven by the human CMV promoter and utilizes simian virus 40 (SV40) polyadenylation elements. The entire expression cassette is situated within an intron in the adenovirus E1B region immediately downstream of the 19-kD E1B gene. FGR expresses the wild-type adenovirus type 5 E1A and 19-kD E1B proteins. Two premature translation stop codons were placed in the 55-kD E1B gene that would result in the production of a truncated 78-amino acid protein. This truncated protein does not contain the p53 binding domain (amino acids 215–354, Yew and Burke, 1992; Kao *et al.*, 1990), and therefore cannot interact with p53. FGR also contains a 2.68-kb deletion in the E3 region (bases 28,133–30,181).

To demonstrate that FGR had the expected properties, virus-infected DU145 prostate adenocarcinoma cells were examined

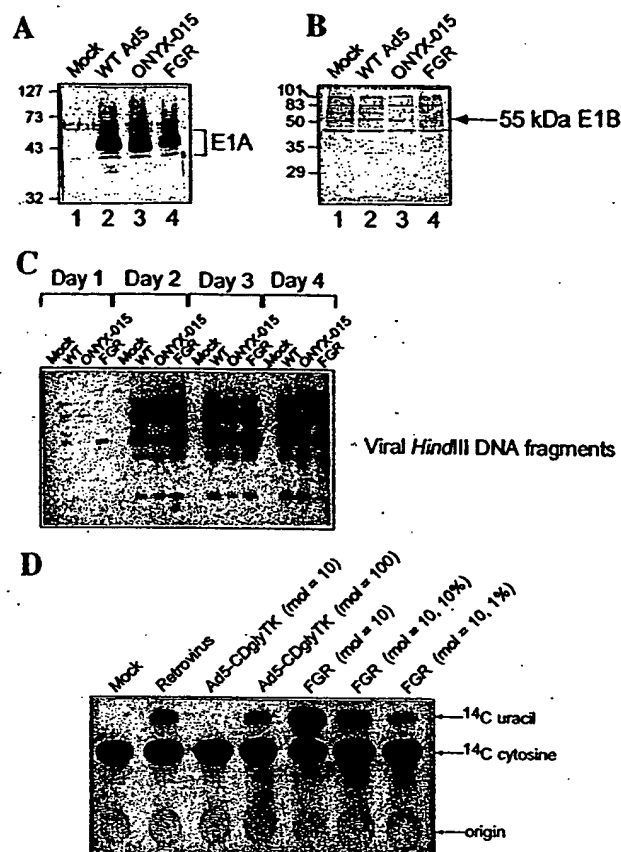
for expression of the E1A and 55-kD E1B proteins, virus replication, and CD activity (*i.e.*, expression of the CDglyTK fusion protein). FGR produced similar amounts of the E1A proteins as wild-type adenovirus type 5 and ONYX-015 (Fig. 2A). However, like ONYX-015, it failed to express the 55-kD E1B protein (Fig. 2B). FGR replicated in DU145 cells with kinetics similar to that of wild-type adenovirus and ONYX-015 (Fig. 2C); however, it and ONYX-015 replicated slightly slower than wild-type virus in most other tumor cell types examined (*e.g.*, U251, HT-29; not shown). The titer (pfu) of virus produced in one cycle "burst" assays correlated well with the steady-state levels of viral DNA observed in the Southern blots (not shown). The replicative capacity of FGR subsequently resulted in high expression of the CDglyTK fusion protein as determined by CD assays. Seventy-two hours post-infection, FGR expressed the CDglyTK protein at a level 50-fold greater than a stably, retrovirally transduced cell line and 300-fold greater (after correcting for differences in moi) than cells infected with Ad5-CDglyTK, a replication-defective version of FGR (Fig. 2D). This clearly illustrates the advantage of a replication-competent adenovirus over a nonreplicating adenovirus (*i.e.*, greater therapeutic gene expression per dose).

To demonstrate that FGR, like ONYX-015, preferentially



**FIG. 1.** Schematic representation of the E1 region of FGR. All numbers indicate base positions of the wild-type adenovirus type 5 sequence. The natural 19-kD E1B translation stop codon and the two engineered premature stop codons in the 55-kD E1B reading frame are indicated. Horizontal arrows indicate E1A, E1B, and CMV transcription initiation sites. The caret indicates the intron in the E1B region into which the CD/HSV-1 TK expression cassette was cloned. Solid black boxes in the CD/HSV-1 TK fusion gene represent the CMV promoter or SV40 polyadenylation elements, and the open box indicates the region coding for the polyglycine linker. SD, Splice donor site.





**FIG. 2.** Characterization of FGR. **A.** Expression of adenovirus E1A proteins. DU145 cells were either mock-infected, or infected with virus at an moi of 10. Cells were harvested 18 hr later and examined for E1A expression by Western blotting. The migration of the E1A proteins is indicated. Numbers on the left indicate molecular weight markers in kilodaltons. **B.** Expression of adenovirus 55-kD E1B protein. DU145 cells were either mock-infected, or infected with virus at an moi of 10. Cells were labeled with [ $^{35}$ S]methionine, and the 55-kD E1B protein was immunoprecipitated from cell extracts. Immunoprecipitates were analyzed on a SDS-10% polyacrylamide gel. The migration of the 55-kD E1B protein is indicated. Numbers on the left indicate molecular weight markers in kilodaltons. **C.** Comparison of viral replication. DU145 cells were either mock-infected or infected with virus at an moi of 10. Cells were harvested every day thereafter, and low-molecular-weight DNA was isolated. DNA was digested with *Hind* III and subjected to Southern blot analysis. The  $^{32}$ P-labeled probe contained adenovirus type 5 sequences from base 1,340 to the right end and detects all but the left hand-most *Hind* III fragment of the viruses. **D.** Comparison of CD expression among various viruses. DU145 cells were either mock-infected, or infected with FGR at an moi of 10 or a replication-defective version of FGR (Ad5-CDglyTK) at mois of 10 and 100. Cells were harvested 72 hr later and examined for CD activity. All CD assays contained 50  $\mu$ g of protein, except for FGR (moi = 10, 10%) and FGR (moi = 10, 1%), which contained 5  $\mu$ g and 0.5  $\mu$ g, respectively. For comparison, CD expression in adenovirus-infected cells is compared to a pooled, G418-selected DU145 cell line (retrovirus) that expresses the CDglyTK fusion protein from a stably integrated murine provirus (Rogulski *et al.*, 1997a). The [ $^{14}$ C]uracil product and [ $^{14}$ C]cytosine substrate are indicated. The transduction efficiency of DU145 cells at the various mois are as follows: Ad5-CDglyTK, moi = 10, 20–30%; Ad5-CDglyTK, moi = 100, 100%; FGR, moi = 10, 20–30%.

killed tumor cells lacking functional p53, DU145 (human prostate adenocarcinoma, mutant p53), U251 (human glioblastoma, mutant p53), and MRC-5 cells (human diploid fibroblasts, wild-type p53) were infected with graded doses of ONYX-015 and FGR and the resulting cytopathic effect was scored 9 days later (Fig. 3). FGR lysed DU145 and U251 cells with efficiencies identical to ONYX-015. DU145 cells were more sensitive to both viruses than U251 cells. By contrast, both viruses produced only minimal cytopathic effects with MRC-5 cells (at high moi and after prolonged periods). Thus, FGR (and ONYX-015) is able to kill tumor cells containing mutant p53 under conditions where it demonstrates no cytopathic effect on normal cells.

#### *Enhancement of the viral cytopathic effect by the CD/5-FC and HSV-1 TK/GCV suicide gene systems*

To determine whether the CD/5-FC and HSV-1 TK/GCV suicide gene systems could enhance the viral cytopathic effect, cells were infected with FGR and exposed to increasing concentrations of 5-FC, GCV, or both 5-FC and GCV. The cytopathic effect was scored qualitatively by staining cells 5–9 days post-infection and quantitatively by clonogenic assays. For U251 cells, both the CD/5-FC and HSV-1 TK/GCV systems increased the cytopathic effect of the virus in a prodrug concentration-dependent manner (Fig. 4A, top). Whereas FGR alone consistently produced about 1 log cell kill in clonogenic assays, 5-FC ( $\geq 50$   $\mu$ g/ml) and GCV ( $\geq 0.5$   $\mu$ g/ml) each increased the cytotoxic effect by an additional log (Fig. 4B). The effect of FGR on DU145 cells was so potent that it masked the cytopathic effects of the suicide gene systems (not shown). Therefore, we performed similar experiments using another well-characterized human prostate adenocarcinoma cell line, PC-3 (null p53), which is rather resistant to both FGR and ONYX-015. Similar to U251 cells, the CD/5-FC suicide gene system dramatically increased the observed cytopathic effect relative to that of FGR alone (Fig. 4A, middle). However, no increase was observed with the HSV-1 TK/GCV system up to concentrations of 1.0  $\mu$ g/ml GCV. Thus, at least one suicide gene system was able to elicit a pronounced cytopathic effect with a tumor cell line that is very resistant to these lytic viruses. Similar results were obtained with the human glioblastoma line LN-Z308 (null p53), which also proved to be resistant to these lytic viruses (not shown). Importantly, when used independently, neither suicide gene system had any significant effect on MRC-5 cells except at the highest (100  $\mu$ g/ml) 5-FC concentration used (Fig. 4A, bottom).

#### *CD/5-FC and HSV-1 TK/GCV suicide gene systems markedly inhibit viral replication*

Because both the CD/5-FC and HSV-1 TK/GCV systems exert their cytotoxic effect by inhibiting DNA replication either directly (HSV-1 TK/GCV) or indirectly (CD/5-FC), we examined whether these systems could be used to inhibit viral replication. Shortly after FGR infection, cells were plated in increasing concentrations of prodrugs and adenoviral DNA was isolated 2 days later and subjected to Southern blot analysis (Fig. 5). Both suicide gene systems markedly inhibited viral replication. However, in all cell lines tested (U251, DU145, HT29), the HSV-1 TK/GCV system was approximately 1,000-fold more effective on a prodrug concentration basis. This may, in part, be explained

by the fact that the HSV-1 TK/GCV system inhibits DNA replication directly at the level of chain elongation, whereas the CD/5-FC system depletes dTMP precursor pools. Interestingly, a dramatic enhancement of the viral cytopathic effect was observed (Fig. 4A) even at prodrug concentrations that markedly inhibited replication of the virus (for U251 cells:  $\geq 50$   $\mu\text{g/ml}$  5-FC and  $\geq 0.1$   $\mu\text{g/ml}$  GCV). These results argue that in such cases, the cytopathic effect due to the suicide gene systems is much more potent than that of the virus itself.

#### Radiosensitization by FGR and prodrugs

Previously, we demonstrated that the CD/5-FC and HSV-1 TK/GCV suicide gene systems could, independently, potentiate the killing effects of radiation *in vitro* (Kim *et al.*, 1994, 1995, 1997; Khil *et al.*, 1996; Rogulski *et al.*, 1997a) and *in vivo* (Rogulski *et al.*, 1997b) (*i.e.*, radiosensitivity). Even greater radiosensitivity was observed when both suicide gene systems were used together (Rogulski *et al.*, 1997a). A tremendous strength of the combined suicide gene therapy/radiotherapy approach is that because neither prodrug (5-FC and GCV) is toxic and it is possible to focus a radiation beam on a very small area in three-dimensional space, it allows for a high degree of tumor cell kill while minimizing damage to normal tissues. Thus, we examined whether these suicide gene systems, when used in the context of a replicating adenovirus, could radiosensitize tumor cells *in vitro*. It is important to note that, because these experiments would employ up to three modalities—viral therapy, suicide gene therapy, and radiotherapy—each of which when used independently under optimal conditions can generate between 1 and 4 logs of cell kill depending on the cell type, it was necessary to use suboptimal amounts of virus and prodrugs to be able to quantify the radiosensitization effect. Thus, the effects described below are likely to represent only a fraction of what could be achieved under optimal conditions.

DUI45 and U251 cells were either mock-infected, or infected with FGR at an moi of 1. Two days later, they were exposed to 40  $\mu\text{g/ml}$  5-FC or 0.1  $\mu\text{g/ml}$  GCV for 24 hr. Cells then received a single dose of  $\gamma$ -irradiation (0–6 Gy) and were immediately replated for clonogenic assays. Nine days later, colonies were scored and cell survival curves generated. A change in the slope of the cell survival curve relative to radiation alone indicates radiosensitization.

Both the CD/5-FC and HSV-1 TK/GCV suicide gene systems radiosensitized DUI45 and U251 cells (Fig. 6A,B), yielding sensitivity enhancement ratios (SERs) in the range of 1.6–3.1. By contrast, FGR alone resulted in no radiosensitization, indicating that the combined toxic effects of the replicating virus and radiation are additive. Thus, even under suboptimal conditions, both suicide gene systems could, in a synergistic manner, enhance the killing effects of radiation when expressed in the context of a replicating adenovirus.

#### FGR infection of tumors *in vivo*

To estimate the efficiency at which FGR could infect and spread in tumors, HT-29 tumors (human colon adenocarcinoma, mutant p53) were injected with FGR and tumors

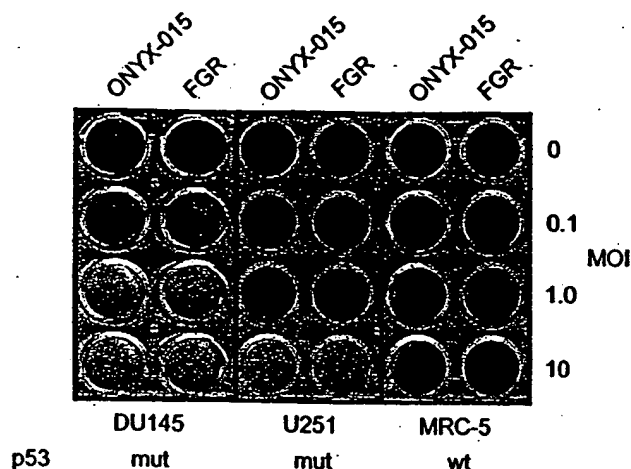
were excised and examined for CD expression by immunofluorescence. Although the level of CD expression varied from cell to cell, the results demonstrated that approximately 20–30% of tumor cells expressed the CD gene product (Fig. 7). As expected, the highest CD expression occurred in cells surrounding the injection site. Similar results were obtained with U251 tumors (human glioblastoma, mutant p53; not shown). Thus, it is possible to achieve significant infection of tumor cells *in vivo* using relatively small amounts of the FGR virus.

## DISCUSSION

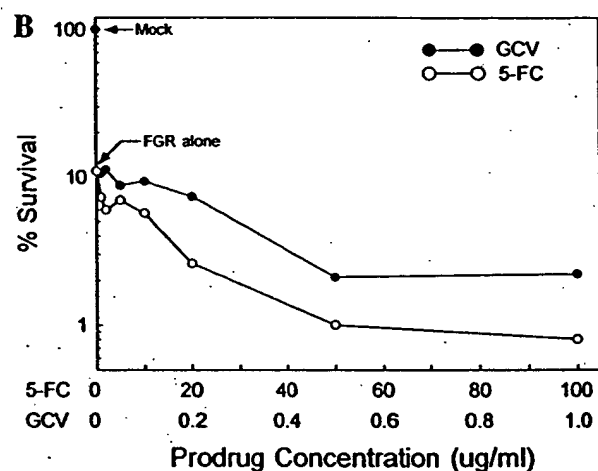
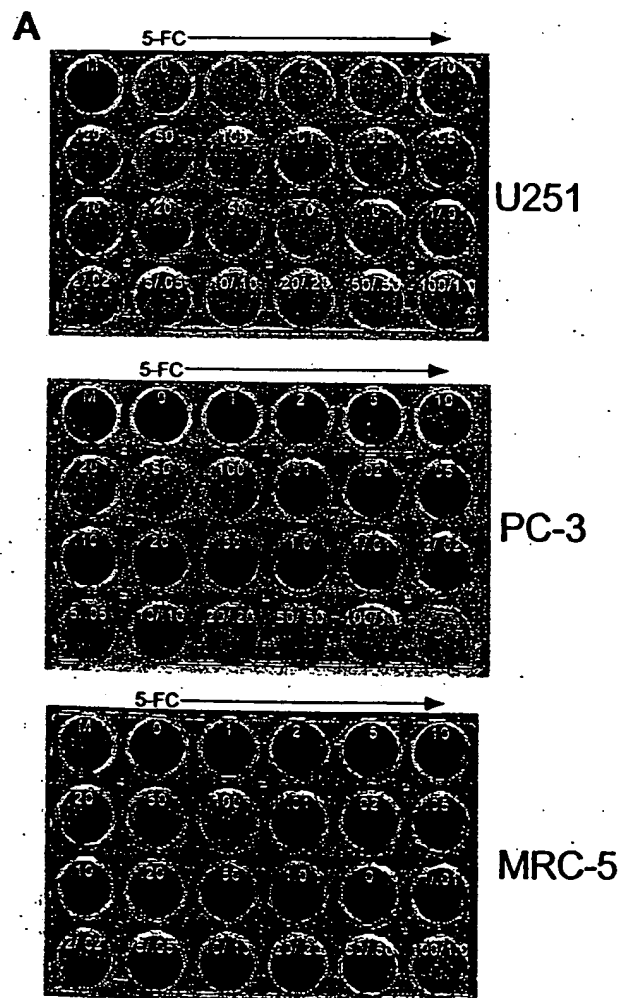
The results presented here demonstrate that suicide gene therapy can enhance the therapeutic effects of viral therapy in a tumor cell-specific manner. The therapeutic effect of these combined modalities can be further enhanced by coupling them with radiotherapy. Although it was not possible to quantify the effectiveness of this three-pronged approach under optimal conditions *in vitro*, it is noteworthy that each modality alone can achieve between 1 and 4 logs of cell kill under optimal conditions depending on the tumor cell type. Thus, even if the combined cytotoxic effects of these modalities was simply additive under optimal conditions, it should be possible to achieve very significant cell kill when these three modalities are used simultaneously. That suicide gene therapy and radiotherapy interact to produce a synergistic enhancement of cell kill may make the combined therapeutic effect even greater. Thus, the three-pronged approach described here (Fig. 8) may prove to be a very powerful means of destroying cancer cells *in vivo*.

Despite its potential, however, improving the effectiveness of a treatment is of no clinical value if there is no significant increase in the therapeutic index, the differential response between tumor and normal tissue. When taking this important point into consideration, the three-pronged approach described here fares well. As demonstrated previously (Bischoff *et al.*, 1996; Heise *et al.*, 1997) and here, the use of an E1B-attenuated adenovirus allows for the preferential targeting of tumor cells lacking functional p53. Thus, the viral cytopathic effect, although not restricted to tumor cells lacking functional p53, is preferentially observed in such cells relative to normal cells. Although the suicide gene systems do not specifically target tumor cells, they do target replicating cells and, importantly, unlike conventional chemotherapy, neither prodrug produces significant systemic toxicity in humans. Finally, with three-dimensional conformal radiotherapy, it is possible to deliver high doses of radiation specifically to a tumor site while minimizing the exposure to surrounding tissues. On the basis of these premises, we believe the three-pronged approach described here may generate a significant increase in the therapeutic index when used in the clinic.

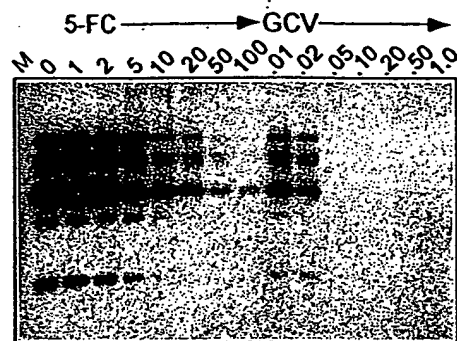
Nearly all gene therapy protocols undergoing clinical trials today are limited by low *in vivo* gene transduction efficiencies. Although several approaches using replication-defective retroviruses or adenoviruses have produced encouraging results in animal models, it is unlikely that these approaches will have the gene transduction efficiencies necessary for them to be ef-



**FIG. 3.** Comparison of ONYX-015 and FGR cytopathic effects. Cells were either mock-infected ( $\text{moi} = 0$ ), or infected with virus at the  $\text{mois}$  indicated. Cells were fixed and stained with crystal violet 9 days later.



**FIG. 4.** Enhancement of the viral cytopathic effect by the suicide gene systems. Cells were either mock-infected (M), or infected with FGR at an  $\text{moi}$  of 10. Cells were detached and replated in triplicate at high (A) or low (B) densities in varying concentrations of 5-FC, GCV, or 5-FC + GCV (5-FC/GCV). Cells were fixed and stained with crystal violet 5–9 days later. Numbers at the top of each well in A indicate prodrug concentration in  $\mu\text{g/ml}$ . Results of U251 cell clonogenic assays are shown in B.

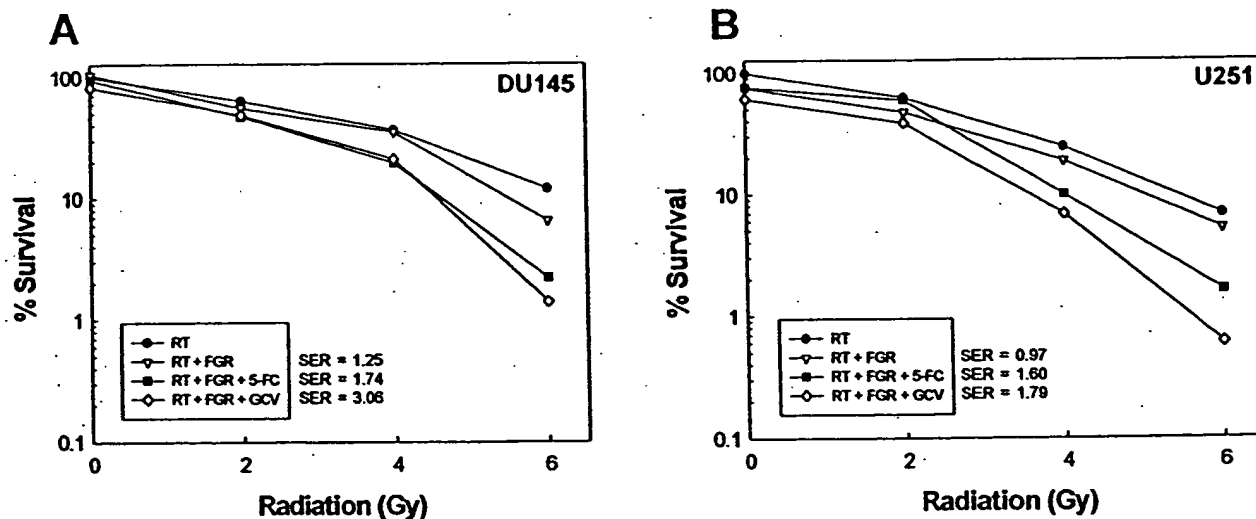
Viral *Hind*III DNA fragments

**FIG. 5.** Inhibition of virus replication by suicide gene systems. Cells were either mock-infected (M), or infected with FGR at an moi of 10. Cells were detached and replated in varying concentrations of prodrugs. Cells were harvested 48 hr later for isolation of low-molecular-weight DNA. DNA was digested with *Hind* III and analyzed by Southern blotting as described in Fig. 2C.

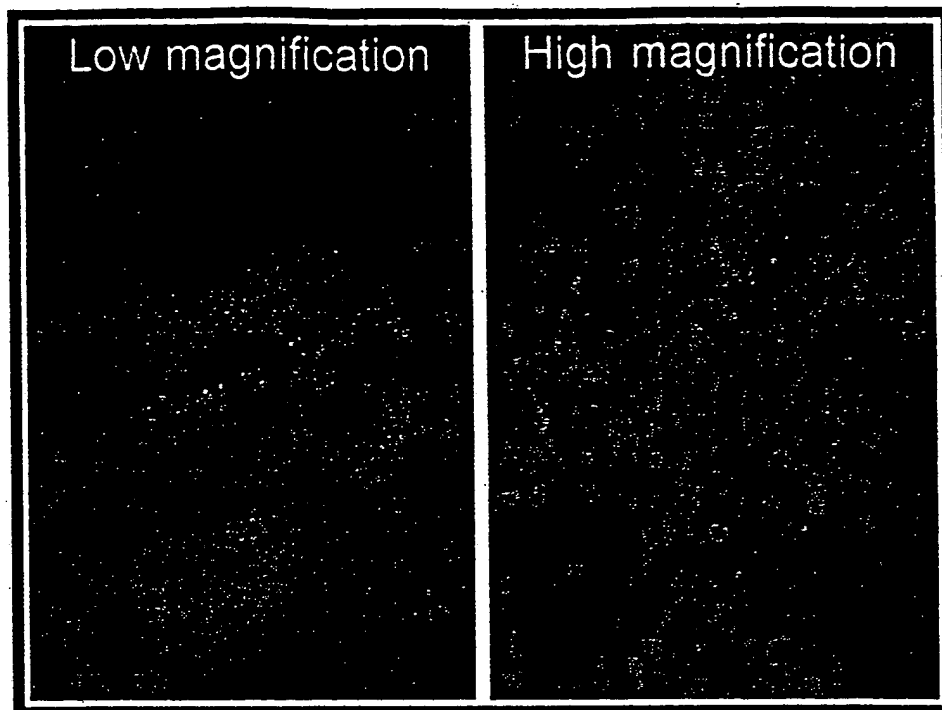
fective against the large tumoral masses commonly observed in human cancer patients. Moreover, because few human cancers are curable with a single modality, it has been our tenet that the promise of cancer gene therapy will be realized only when used in combination with other modalities, such as the ones described here. We believe the three-pronged approach described here may improve not only the specificity (discussed above), but also the efficacy, of the cancer gene therapy approach. Relative to a replication-defective virus, the replication capacity of FGR should result in a markedly greater efficiency of gene transfer *in vivo* as well as higher transgene expression per tumor cell. Indeed, we have observed that FGR is able to produce up to

2,000-fold more CD activity in infected cells than a replication-defective version from which FGR was derived (after correcting for differences in mois). This should result in a greater therapeutic effect per dose than that obtainable with replication-defective viruses. It is possible that the high *in vivo* gene transduction efficiencies observed with replication-competent adenoviruses may enable this approach to be efficacious against disseminated disease (Heise *et al.*, 1997).

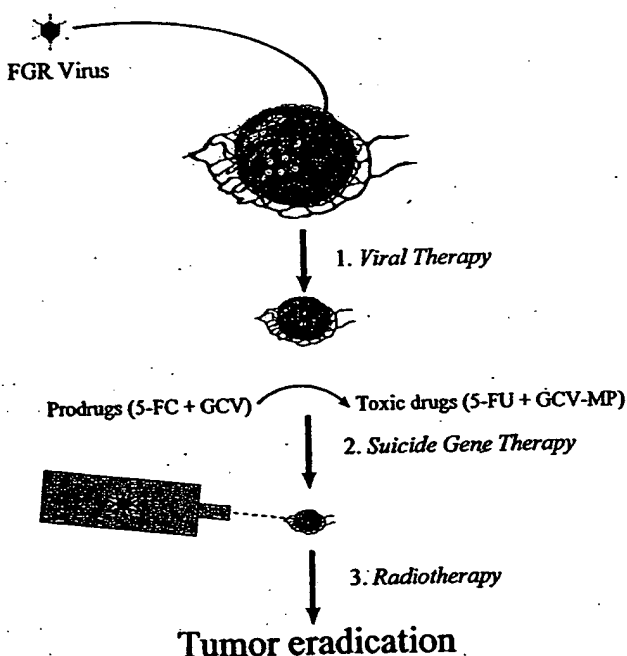
Another potential strength of the approach described here is that both suicide gene systems proved to be effective at inhibiting viral replication *in vitro* at prodrug concentrations that are safely achievable in humans. Although it is uncertain



**FIG. 6.** Radiosensitization by the suicide gene systems. DU145 (A) and U251 (B) cells were either mock-infected (●), or infected with FGR at a moi of 1. Forty-eight hours later, cells were incubated in growth medium without (▽), or with, prodrugs at 40  $\mu$ g/ml 5-FC (■) or 0.1  $\mu$ g/ml GCV (◇) for 24 hr. Cells were then exposed to a single dose of  $\gamma$ -irradiation (0–6 Gy) and replated in triplicate for clonogenic assays. Colonies were fixed, stained with crystal violet, and counted 7–10 days later. The SER represents the ratio between the  $D_0$  of radiation alone (RT) and the  $D_0$  for radiation plus FGR without (RT + FGR), or with (RT + FGR + 5-FC, RT + FGR + GCV), prodrugs. The radiosensitization experiments were performed twice and the SERs varied less than 10% between experiments.



**FIG. 7.** FGR infection of tumor cells *in vivo*. HT-29 tumors ( $0.2 \text{ cm}^3$ ) were injected with  $10^8$  pfu ( $50 \mu\text{l}$ ) of FGR on 5 consecutive days. Two days after the last injection, tumors were excised, sectioned, and processed for immunofluorescence. Samples were photographed at a magnification of  $25\times$  (left) and  $125\times$  (right). Cells that are positive for CD expression appear red. Nuclei were stained with DAPI and are blue.



**FIG. 8.** Schematic diagram of three-pronged approach. The FGR virus itself (prong 1) replicates in and preferentially kills tumor cells expressing mutant p53 resulting in significant, and selective, tumor cell destruction. Co-utilization of the CD/5-FC and HSV-1 TK/GCV suicide gene systems (prong 2) markedly enhance the therapeutic effects of the FGR virus and, importantly, sensitize tumors to radiation (prong 3). The replication potential of the FGR virus enhances both the efficiency of gene transduction (percentage of tumor cells (infected) as well as the steady-state level of transgene expression per cell.

whether uncontrolled viral spread will prove to be a drawback of this approach, having the potential to eliminate the virus, if necessary, is an asset. These observations also make it likely that following injection of the virus, both the timing and dose of prodrug administration will be critical parameters in determining the therapeutic outcome. Indeed, results from our ongoing animal studies support this notion. Thus, inclusion of the CD/HSV-1 TK fusion gene in a lytic, replication-competent adenovirus may not only improve the efficacy, but also the safety, of this cancer gene therapy approach.

There is perhaps one additional advantage of the three-pronged approach described here. Although the use of an E1B-attenuated adenovirus, such as ONYX-015, allows for the preferential destruction of tumor cells lacking functional p53, its inability to destroy tumor cells selectively with normal p53 is a weakness. Moreover, we have observed that some human tumor cell lines are resistant to these lytic viruses. These weaknesses may prove to be significant as many human tumors show intratumoral heterogeneity with respect to p53 status (Mirchandani *et al.*, 1995; Yang *et al.*, 1996) and the efficacy of this approach may vary widely among tumor types. Thus, a better approach might be one that could selectively destroy tumor cells lacking, or containing, functional p53, and yet inflict minimal damage on surrounding normal cells. Indeed, FGR, when used in conjunction with prodrug and radiation therapy, may meet such criteria. It has been previously demonstrated that tumor cells with normal p53 status are more sensitive to the cytotoxic effects of chemotherapeutic drugs, such as 5-FU, and radiation than tumor cells lacking functional p53 (Lowe *et al.*, 1993, 1994). The heightened sensitivity of tumor cells with normal p53 is thought to be due to their increased propensity to undergo p53-mediated apoptosis following injury.

Because our three-pronged approach employs both chemotherapy and radiotherapy, it is possible that FGR, when combined with these modalities, may selectively destroy both tumor cell types. Whereas the viral cytopathic effect should selectively target tumor cells lacking functional p53, the suicide gene systems, via their bystander effects, and radiotherapy should preferentially target those containing functional p53. Importantly, this approach should inflict minimal damage on surrounding normal cells because such cells are less likely to be replicating (and therefore resistant to suicide gene therapies) and normal cells tend to undergo p53-mediated growth arrest, not apoptosis, following insult by chemotherapeutic drugs and/or radiation (Lowe *et al.*, 1993). Thus, utilization of the suicide gene systems should not significantly weaken the specificity of the "tumor-specific" E1B-attenuated adenoviruses. We have already demonstrated that FGR, in conjunction with 5-FC prodrug therapy, is able to kill effectively tumor cells that are resistant to lytic viruses such as ONYX-015 and FGR.

Thus, it is likely that the three-pronged approach described here will prove to be a significant improvement over ONYX-015 because the three modalities may target different tumor cell types or subpopulations, which, in turn, should expand the spectrum of human tumors that it will be effective against. Indeed, our ongoing studies in animals have confirmed that the addition of double suicide gene therapy significantly improves the antitumor effects (*i.e.*, tumor growth delay and tumor cure) of E1B-attenuated, replication-competent adenoviruses.

## ACKNOWLEDGMENTS

We thank our colleagues for their help and insight. We thank Dr. C. Heise (ONYX) for providing wild-type adenovirus type 5 and ONYX-015. This work was supported by grants from the National Institutes of Health and ACS to SOF and JHK (CA64295, CA75456, CA64323, EDT-110).

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|                 |   |                   |                     |
|-----------------|---|-------------------|---------------------|
| Application of: | Wold et al.                               | Group No.:        | 1632                |
| Serial No.:     | 09/351,778                                | Atty. Docket No.: | 66153-7775          |
| Filed:          | 07/12/1999                                |                   |                     |
| For:            | Replication-Competent Anti-Cancer Vectors | Examiner:         | Priebe, Scott David |

**DECLARATION, PURSUANT TO 37 C.F.R. §1.131,**  
**OF WILLIAM S.M. WOLD, Ph.D., ANN E. TOLLEFSON, Ph.D., KONSTANTIN**  
**DORONIN, Ph.D. AND KAROLY TOTH, D.V.M.**

We, William S.M. Wold, Ph.D., Ann E. Tollefson, Ph.D., Konstantin Doronin, Ph.D. and Karoly Toth, D.V.M., declare and state as follows:

1. All of the statements made herein of our own knowledge are true and all statements made on information and belief are believed to be true.
2. We are co-inventors of the inventions described and claimed in the above referenced patent application, filed July 12, 1999.
3. We conceived of a recombinant adenovirus vector which is replication-competent in neoplastic cells and which overexpresses an adenovirus death protein prior to March 3, 1997. We worked diligently, continuously and uninterrupted from prior to March 3, 1997, until we reduced the recombinant adenovirus to practice. The information contained in the documents of the attached exhibits was contemporaneously recorded on the dates indicated therein. Further, those exhibits are



true copies of the originals except to the extent that certain sensitive dates have been redacted. All noted dates that have not been redacted are accurate.

4. Claim 1 of the '778 application is directed to a recombinant adenovirus vector that is replication-competent in neoplastic cells and that overexpresses adenovirus death protein (ADP).

5. On or before March 3, 1997, we conceived of a recombinant adenovirus vector that is replication-competent in neoplastic cells and that overexpresses ADP, as evidenced by the activities set forth below. Unless otherwise noted, all of the documents referred to in the bullet point sections below are dated prior to March 3, 1997:

- Our early work focused on the E3 transcription unit (also referred to as the E3 region) of Adenovirus (Ad), and understanding the function of the various proteins encoded by that region. We observed that Ad comprising an ADP (11.6K) mutation (i.e. a mutation in the gene coding for ADP) developed plaques slower, and that those plaques were smaller, than Ad with wild type levels of ADP expression (see Exhibit A, page 1 [A1], where dl712 is an ADP mutant and Ad5 is a wild-type adenovirus).
- In addition, we observed that some mutant Ads that have portions of the E3 region deleted, other than the gene for ADP, produced larger plaques than the wild-type Ad. See page 2 of Exhibit A (page A2), which is a notebook page memorializing these observations. This page shows that dl753 produced larger plaques than wild-type.
- Plaque development assays indicated that ADP mutant Ad produced plaques at different rates than wild type Ad (see pages A3-A17, which are plaque assay results which show that dl753 and dl732 produce plaques faster than wild type Ad, and ADP deletion mutants form plaques slower than wild-type). Note that "rec700" represents wild-type Ad, and "dl 753" and "dl 732" are adenoviruses that have deletions in the E3 region [not in the open reading frame for ADP] that result in increased synthesis of ADP; and dl712, dl801, dl742, dl708 and dl7001 have deletions in the coding region for ADP that preclude synthesis of functional ADP.
- Gel electrophoresis studies were carried out which indicated that E3 deletion Ad produced higher levels of ADP (see Exhibit A, pages A18-A20, which are photocopies of those gels that show that dl753 (an E3 deletion mutant) produces more ADP than rec700 (which produces wild-type levels of ADP)).
- Immunofluorescence assays were carried out that confirmed these findings that E3 deletion mutants produce more ADP than wild-type (See Exhibit A, pages A21-A25, where dl732 and dl753 are E3 deletion mutants that overexpress ADP, dl712 is an E3 mutant in which the gene for ADP is deleted, and rec700 is wild-type).

- In virus release assays, we observed that the ADP mutants that had large plaques released more virus than both wild-type Ad and ADP mutants that had small plaques (A26).

- This early work led us to hypothesize that ADP is required for cell lysis, and release of Ad. We also hypothesized that over-expression of ADP may cause cell lysis more rapidly, and therefore, that vectors that overexpress ADP may be useful as anti-cancer agents.

- A proposal, entitled "Adenovirus E3-11.6K Protein as a Cell Death-Promoting Agent," was sent to Dr. Rae Lyn Burke of Chiron Corp. That Proposal is submitted herewith as Exhibit B. At page 7 of that proposal, the concept of overexpressing ADP (11.6K) is discussed. Further, at page 4 the KD/GZ class of adenovirus vectors are described, where it is stated that "[t]he nondefective vectors generally have the E3 transcription unit deleted and replaced with the transgene" [e.g., ADP]. The concept that deletion of the E3 transcription unit and insertion of the ADP gene would result in overexpression is based on the understanding that deletion of most or all of the E3 genes other than the ADP gene facilitates overexpression of ADP mRNA by reducing competition for splicing of the major late pre-mRNAs. That concept is put forth in the instant specification at page 15, lines 12-17. That concept is also discussed in the proposal at page 7, where it is stated that "...we will determine whether 11.6K overexpressed during early stages of infection can promote cell death. The 11.6K gene will be built into our mutant, dl7001, which lacks the entire E3 region but expresses all other adenovirus genes... Cells will be infected with the dl7001-11.6K vector...and cell death will be monitored."

6. Prior to March 3, 1997, we constructed a recombinant adenovirus vector that is replication-competent in neoplastic cells and that overexpresses ADP. Specifically, KD1 is a recombinant adenovirus vector that is replication-competent in neoplastic cells and that overexpresses ADP. KD1 was constructed by cotransfecting dl1101/1107 DNA that had been digested with EcoRI and plasmid p54 DNA comprising the ADP gene into 293 cells. DNA from resulting plaques was confirmed as KD1 by PCR. The following activities, which occurred prior to March 3, 1997 are related to the construction and evaluation of ADP overexpressing vectors generally, and specifically KD1:

- High titer dl1101/1107 stocks were expanded for the purpose of examining whether this virus with a mutation in the E1A gene would grow well on cancer cell lines and not on non-cancerous cell lines, and also for the purpose of deleting the E3 region of an Ad strain and inserting the ADP from Ad5. (See Exhibit D1) (dl1101/1107 serves as the "backbone" of the KD strains described in the 09/351,778 application, see page 22, Table 1).

- High titer dl1101/1107 stocks were confirmed. (D2-D3).
- The EcoRI-A fragment of Ad5 containing the DNA sequence encoding ADP was purified. (D4)
  - Various deletions and insertions were made in the backbone adenoviral E3 region by PCR. For example, in the construction of KD1, the ADP gene was isolated from the Ad5 genome by PCR using the primers named KD6 and KD7 that added PaeI restriction sites to the DNA fragment containing the ADP gene. (E3, E4 and E9). This ADP-containing PCR fragment was cloned into a PaeI site in the plasmid named pL2 to produce the shuttle plasmid named p54 (F7-F8) (D9). Another name for plasmid pL2 is pLKHE2A + Bam → end + Cla. The sequences for primers KD6 and KD7 are presented in Appendix E1, E3, E4 and E9 and are described in the examples in the '778 application. These primers as well as other primers were purchased from Gibco BRL. Exhibit E comprises order sheets and certificates of analysis for a number of those primers, along with contemporaneous notes that describe the primers. (E1-E10)
  - Growth curves to assess whether dl1101/1107 is attenuated for growth as compared to dl309 in HEL299 cells. (I1-I31)
  - Various plasmids used to construct the KD vectors were sequenced. (F1-F8)
  - The DNA sequence of the ADP gene inserted into plasmid p54 was determined using the KD6 and KD7 primers (F7-F8)
  - The ADP gene was successfully subcloned into the shuttle plasmid vector, culminating in the isolation of the KD1 shuttle plasmid vector. (Exhibit F5-F6) (D9)
  - dl1101/1107 DNA that had been digested with EcoRI was cotransfected into 293 cells together with plasmid p54 DNA. (D39)
  - Seven putative KD1 plaques from the above transfection experiment were observed. (C1) (KD) (WW) DNA from these plaques is confirmed as KD1. (C6)
  - New DNA was prepared from cells infected separately with viruses isolated from ten plaques that resulted from the above co-transfection experiment. (C6)
  - The above DNA was analyzed by restriction enzyme digestion and PCR. Three of the plaques were shown to contain the E3 region from plasmid p54; that is, three of the plaque isolates were KD1. (C9) (D48-49)
  - A second cotransfection experiment was performed using EcoRI-digested dl1101/1107 DNA plus plasmid p54 in a repeat attempt to construct KD1. (D47). On 3/6/97 the Hirt supernatant DNA from four plaque isolates had been analyzed by PCR;

three of the plaque isolated had the E3 region of KD1. (D52) (C16). Thus, prior to March 3, 1997, KD1 had been constructed in two separate experiments.

7. From the time the KD1 vector was made, we continued to work on construction and characterization of additional replication competent vectors that overexpress ADP, as evidenced by the activities listed below. This work eventually led to the KD3, GZ1 and GZ3 vectors described in the specification of the '778 patent, particularly in the Examples. Our experiments show that KD1 overexpresses ADP. Specifically, on or about May 20, 1997, we show that KD1 develops plaques faster than dl309, which expresses wild-type levels of ADP. Faster plaque development is correlated with overexpression of ADP. Based on the plaque development assay, we concluded that KD1 overexpresses ADP. In addition to the activities listed below, certain activities occurred on a continual basis. Those activities include, among others, maintenance of stocks of human cell lines used in evaluating the vectors; maintenance of bacterial cell cultures used to generate stocks of the various plasmids used to construct the claimed vectors; experiments such as virus growth curves, virus plaque assays, virus spread assays, virus induced cell death assays, all of which require multiple days to complete; lab meetings and informal discussions of progress and strategies for future experiments. The following activities are evidence of our continuous efforts:

**3/3/97-3/7/97:**

- From prior to March 3, 1997 through at least March 7, 1997, plaque assays of KD1 (also referred to as 544 in the attached exhibits) continued to progress and observations were made. Also, stocks of cells comprising the recombinant virus with the E3 region deleted and ADP inserted were maintained. (C15-16)

- On 3/6/97 the Hirt supernatant DNA from four plaque isolates from the second cotransfection experiment performed using EcoRI-digested dl1101/1107 DNA plus plasmid p54 had been analyzed by PCR; three of the plaque isolated had the E3 region of KD1. (D52) (C16).

- Growth curves with dl1101/1107 and dl309 on HEL299 cells or WI38 cells continue. (I32)

**3/7/97-3/14/97:**

- Plaque assays of the KD1 vector continued. (C19)
- Virus growth assays continued on WI38 cells to assess the growth of dl1101/1107. (C20)
- Assays continue to assess dl1101/1107 and dl309 in growth arrested vs. growing HEL299 cells. (C21, I33-34)
- Initial stocks of the KD1 virus had been prepared. (C19)

**3/14/97-3/21/97:**

- The E4 promoter region of dl1101/1107 was sequenced to help in constructing an E4 promoter substituted virus. (C22)
- Virus growth assays continued on WI38 cells to assess the growth of dl1101/1107. (C23)
- Sequencing of plasmids used to construct the KD vectors continued using various primers. (F9-F12)
- Assays continued to assess dl1101/1107 and dl309 in growth arrested vs. growing HEL299 cells. (C24-25)

**3/21/97-4/4/97:**

- HEL299 growing and growth arrested cells are infected with dl1101/1107 and dl309 and virus growth assays are in progress. (C26, I35-36)
- dl1101/1107 extracted from WI38 permissive cells is titered. (C27)

**3/21/97-4/11/97:**

- dl1101/1107/EcoRI and p101 or p111 are cotransfected and cell cultures observed to identify plaques that may comprise the KD2 and KD3 vectors. (C28)

**4/4/97-4/11/97:**

- Growth assays with dl1101/1107 and dl309 continue in HEL299 cells. (C29)
- Titer experiments continue with dl1101/1107 extracted from WI38 cells. (C30)

**4/11/97-4/18/97:**

- Plaques with putative KD2 and KD3 virus are observed, and Hirt preps of DNA from those plaques are made. (C31)

- Titer experiments continue with dl1101/1107 extracted from WI38 cells, and preliminary data are collected. (C32)

- Growth curve experiments with dl1101/1107 and dl309 on growth arrested and growing HEL299 cells are continued and data are compiled. (C34-35)

#### 4/18/97-4/25/97:

- PCR and restriction enzyme digestions are done to confirm KD2, and Hirt DNA preps are made for putative KD3 plaques. (C36)

- Titer experiments continued with dl309 and dl1101/1107 extracted from infected WI38 cells. (C37-39)

- Growth curve experiment was started with dl1101/1107 and dl309 viruses on MCF-7 human breast cancer cells. (C37)

- HEL299 cells infected with dl1101/1107 and dl309 are frozen for growth curve analysis. (C40)

- Sequencing of plasmids used to construct KD vectors continued using various primers. (F13-F17)

- Cell culture supernatant stock of KD virus is given to lab technician (SB) for preparation of a high titer CsCl-banded stock. (C36)

#### 4/18/97-5/2/97:

- Titrations of virus extracted from HEL299 cells infected with dl1101/1107 and dl309 continues. (C45)

- Plaque development assay started with KD1 comparing it to dl309 (wild-type E1A, normal ADP expression), dl1101/1107 (E1A mutation, wild-type ADP expression), pm734.1 (wild-type E1A, no ADP expression), and dl7001 (wild-type E1A, no ADP gene). The cell lines used were 293 and A549 cells. (C41) (D90-D92). The results of this plaque assay are shown graphically at C56. The KD1 and dl309 plaques developed approximately 2 to 3 days faster than did the plaques of dl1101/1107. The fact that KD1 plaques developed more rapidly than those of dl1101/1107 indicates that KD1 overexpresses ADP.

- SB (lab technician) obtained the KD1 virus from KD and began experiments to expand the amount of virus. (K21)

**4/25/97-5/2/97:**

- KD3 vector (referred to as dlE3Xba + ADP) was confirmed by PCR and restriction enzyme digestion. (C41)
- Experiments to expand KD1 continue. (K22 K23)

**5/2/97-5/9/97:**

- Stocks of dlE3 + ADP were grown and monitored for plaque development. Also, cells transfected with KD1, KD2 and KD3 are monitored for development of plaques relative to dl309. (C47, C49)
- Growing and growth arrested dl1101/1107 infected HEL299 cell extracts are titred for growth curve. (C50)
- Expansion of KD1 continues. (K24) (C49)

**5/9/97-5/23/97:**

- Plaque development assays continue, to asses KD1, KD2 and KD3 relative to wild-type. (C51)
- Plaque assays to assess expression of ADP by KD1 relative to several viruses with wild-type E1A genes continue. Fast plaque formation by KD1 indicates ADP overexpression. (H2-H3)
- Growth curves with dl309 and dl1101/1107 extracted from HEL299 cells continue, and data is collected at various intervals. (C52-53)
- Sequencing of primers used to construct KD vectors continued. (F18-F19)
- Titering of dl309 DNA extracted from 293 cells is initiated. (I37)
- Growth curves with dl309 and dl1101/1107 extracted from HEL299 cells continue. (I38)
- Expansion of KD1 continues. (K25) (AT)

**5/13/97-6/2/97:**

- Plaque assay to assess KD1. Results indicate that KD1 overexpresses ADP. (K25-K28)

- Expansion of KD1 continues. (K26)

**5/20/97-6/8/97:**

- Plaque assays of various viruses including KD1 and dl751. (H4-H10). The plaque development curve indicates that KD1 formed plaques as well as or better than dl751, a virus that overexpresses ADP. This suggests that the virus had good ADP expression and function. (K27-28) (H4-H9). KD1 was plaque assayed along with dl751 (which expresses higher than normal quantities of ADP), two preparations of dl707 (a mutant in which a portion of ADP is deleted and which therefore would be expected to have delayed kinetics of plaque formation), and pm734.9 (an ADP mutant with a three amino acid mutation at the C-terminus of ADP [listed as PME on the plaque assay counts]).

**5/23/97-5/30/97:**

- Plaque development assays were monitored and results collected. C56 is a graphical presentation of results indicating that KD1 develops plaques as quickly as dl309 and quicker than dl1101/1107, and that KD2 and KD3 develop plaques more quickly than dl1101/1107. (C55-C58)

**5/30/97-6/27/97:**

- Efforts focused on further characterization of the KD vectors, and improvements on those vectors, such as for example substituting the E4 promoter. Growth curves with dl1101/1107 continued, and Plaque development assays with KD1 indicated that KD1 develops plaques somewhat slower than dl309. (C65-C82)


- Infections were done for immunofluorescence staining for ADP. Viruses used for infection were dl309, KD1, KD2 and KD3. It is noted that KD shows much more staining for ADP than the other viruses (both at 27 and 49 hours post-infection). This is supporting data for the overexpression of ADP by the KD viruses. (H1-H2)

8. As evidenced by the foregoing, we confirmed that KD1 overexpresses ADP by Immunofluorescence assays utilizing an ADP-specific antibody on or about June 23, 1997.

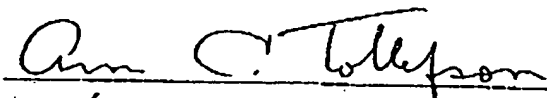
9. We confirmed that KD1 overexpresses ADP by Western blot on or about December 1, 1997. (Exhibit L, pages L2-L4)



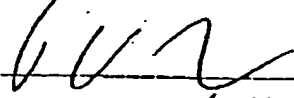
10. I understand that willful false statements and the like are punishable by fine or imprisonment, or both (18 U.S.C. 1001) and may jeopardize the validity of the application or any patent issuing thereon.



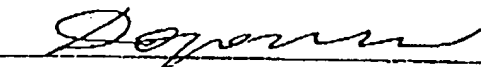
William S.M. Wold, Ph.D.



Ann E. Tollefson, Ph.D.



Karoly Toth, Ph.D. DVM KI



Konstantin Doronin, Ph.D.

## **EXHIBIT A**

+ final counts of

87/972 plaque assays

|                             |              |                      |                                      |
|-----------------------------|--------------|----------------------|--------------------------------------|
| A5<br>① 106                 | 1041<br>① 88 | 801<br>① 120         | (Cumulative)                         |
| ② 83                        | ② 108        | ③ 114                | DME<br>⑤ 0                           |
| ③ 84                        | ③ 95         | ④ 123                | ⑥ 0                                  |
| ④ 13                        | ④ 14         | ⑤ 16                 | ⑦ (all cells dead)                   |
| ⑤ 12                        | ⑤ 14         | ⑥ 12                 | A45 → distinct plqs                  |
| ⑥ 5                         | ⑥ 12         | ⑦ 12                 | 1035 → not as definitive             |
| 1035<br>⑦ omit → cells dead | 918<br>⑦ 78  | 716<br>⑧ 1           | 1041 → more distinct plq             |
| ⑧ 69                        | ⑧ 74         | ⑨ 1                  | 918 → distinct                       |
| ⑨ 68                        | ⑨ 71         | ⑩ 1                  | 305 → distinct                       |
| ⑩ 11                        | ⑩ 14         | ⑪ 0                  | large plaques                        |
| ⑪ 9                         | ⑪ 8          | ⑫ 1                  | 716 → largest, most distinct plaques |
| ⑫ 9                         | ⑫ 10         | ⑬ 0                  |                                      |
| 1038<br>⑬ 110               | 305<br>⑬ 41  | 712<br>⑭ 46          | small plaques                        |
| ⑭ 107                       | ⑭ 37         | ⑮ 57                 | 801 → very small + slow growth       |
| ⑮ 85 (but high cell death)  | ⑮ 41         | ⑯ 52                 | 712 → very small with slow growth    |
| ⑯ 16                        | ⑯ 5          | ⑰ (total cell death) |                                      |
| ⑰ 7                         | ⑰ 1          | ⑱ 6                  |                                      |
| ⑱ 9                         | ⑱ 6          | ⑲ 8                  |                                      |

A2

11.6K → possible related to plaque size  
 + morphology?  
 (tend to vary more  
 in plaque size)

Plaque SizeLarger & distinct

dl 753 (esp.)

dl 754

dl 714

dl 778

Ad 5 (esp.)

dl 799

~~dl 778~~

dl 716 (esp.)

dl 751 (esp.)

dl 718

Smaller / less distinct

most SPY 10.4K mutant

[pm 775 more distinct + larger]

pm 771

dl 713

dl 327 (smaller than  
rec 700 in dl 728)Small

748

712

dl 801

dl 766

dl 742

pm 785

In 724

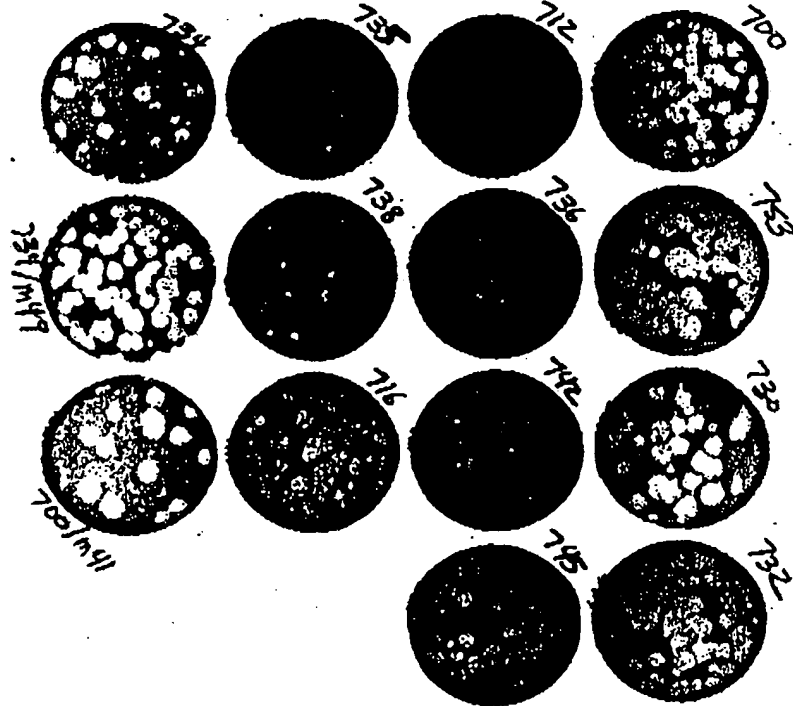
dl 748

dl 706

dl 708

dl 717

dl 7001

**E3-11.6k Small Plaque Mutants**

Early work  
on defining  
ADP function

**EXHIBIT****A3**

EXHIBIT

A4

Plaque Assay

(11.6K Mutants  $\rightarrow$  <sup>t.t.t</sup> plaque assay)

| Virus Prep |                                  | Titre                 | 11. P.S. dishes    |                     |
|------------|----------------------------------|-----------------------|--------------------|---------------------|
|            |                                  |                       | $5 \times 10^{-9}$ | $5 \times 10^{-10}$ |
| ①          | rec 700 (VS188) (9105:5)         | $2.37 \times 10^{11}$ | 2                  | 3                   |
| ②          | dl 742 (860313)                  | $1.77 \times 10^{11}$ | 2                  | 3                   |
| ③          | dl 745 (860313)                  | $2.06 \times 10^{11}$ | 2                  | 3                   |
| ④          | dl 712 (ply 1) (VS204) (911101)  | $1.56 \times 10^{11}$ | 2                  | 3                   |
| ⑤          | dl 730 (39-4) (VS162) (901214)   | $1.73 \times 10^{11}$ | 2                  | 3                   |
| ⑥          | dl 732 (29-1) (AS) (890414)      | $3.4 \times 10^{11}$  | 2                  | 3                   |
| ⑦          | pm 734 (19-2) (890504)           | $6.7 \times 10^{10}$  | 3                  | 2                   |
| ⑧          | dl 735 (34-1) (890425)           | $9. \times 10^{10}$   | 3                  | 2                   |
| ⑨          | dl 736 (0-01) (890414)           | $2.88 \times 10^{10}$ | 3                  | 2                   |
| ⑩          | dl 738 (38-11) (VS171) (910118)  | ?                     | 3                  | 3                   |
| ⑪          | 700/m41 (AS) (920214)            | ?                     | 3                  | 3                   |
| ⑫          | pm 734/m49 (AS) (920402)         | 2                     | 3                  | 3                   |
| ⑬          | dl 716 (ply. 4) (VS125) (900202) | $2.04 \times 10^{11}$ | 1                  | 2                   |
| ⑭          | dl 753 (VS78) (880624)           | $1.42 \times 10^{11}$ | 1                  | 2                   |
| ⑮          | mock                             | -                     | (150)              |                     |

SF-DME for all pre-infection;  
infection with 0.5 ml of dilution at 4:30 pm;  
added 6 ml of overlay at 5:45 pm;

(5/29) added 5 ml of NR overlay; one dish of  
dl 738 ( $5 \times 10^{-9}$  dilution) stopped  $\rightarrow$  discarded

on (5/29) see large distinct plaques on dl 730, dl 732,  
dl 716, dl 753 dishes (+ from dl 115), rec 700 may  
have a few smaller plaques & really do not  
see plaques on other dishes yet.

EXHIBIT

AS

(1/2) plaques marked in red  
(6/3) marked in green

## Counts of (5/25) Plaque Assays

| Sample                             | Dilution                                    | A              | B              | C          | Comments   |
|------------------------------------|---|----------------|----------------|------------|--|
| ① rec 700<br>(V5188)(910503)       | $.5 \times 10^{-9}$<br>$.5 \times 10^{-10}$ | +39 55<br>+8 2 | +35 51<br>+4 2 | -<br>+4 3  | quite large + distinct<br>vary in size.<br>how distinct<br>smaller & less distinct<br>(than 730, 732, 753) |
| ② dl 742<br>(860313)               | $.5 \times 10^{-9}$<br>$.5 \times 10^{-10}$ | +2 3<br>0 0    | +5 2<br>+1 0   | -<br>0 0   | very indistinct<br>very tiny + indistinct  |
| ③ dl 745<br>(860313)               | $.5 \times 10^{-9}$<br>$.5 \times 10^{-10}$ | +20 24<br>+2 5 | +12 22<br>+1 1 | -<br>+2 3  | quite large & slightly<br>larger<br>quite distinct<br>Similar size to rec 700                              |
| ④ dl 712 (ply1)<br>(V5204)(911100) | $.5 \times 10^{-9}$<br>$.5 \times 10^{-10}$ | +8 0<br>+1 0   | +6 0<br>0 0    | -<br>0 0   | no plqs. visible.<br>(at least not definite)<br>very small plaques   |
| ⑤ dl 730 (39-1)<br>(V5168)(901214) | $.5 \times 10^{-9}$<br>$.5 \times 10^{-10}$ | +16 20<br>+1 1 | +22 20<br>+4 3 | -<br>+1 7  | quite large &<br>distinct<br>probably<br>larger than<br>rec 700  |
| ⑥ dl 732 (29-1)<br>(AS)(890414)    | $.5 \times 10^{-9}$<br>$.5 \times 10^{-10}$ | +8 72<br>+3 4  | +12 52<br>+3 1 | -<br>+3 5  | larger + more distinct<br>than dl 730 + dl 753<br>large + very distinct                                    |
| ⑦ pm 734 (1/2)<br>(890504)         | $.5 \times 10^{-9}$<br>$.5 \times 10^{-10}$ | +16 4<br>+4 0  | +8 1<br>+2 0   | +20 5<br>- | probably not very<br>small, but very<br>indistinct<br>than rec 700   |
| ⑧ dl 735 (39-1)<br>(890425)        | $.5 \times 10^{-9}$<br>$.5 \times 10^{-10}$ | +8 6<br>0 0    | +1 0<br>+1 0   | +13 5<br>- | indistinct plaques,<br>size not very definite  |
| ⑨ dl 736 (00-1)<br>(890414)        | $.5 \times 10^{-9}$<br>$.5 \times 10^{-10}$ | +1 1<br>0 0    | +2 1<br>0 0    | +4 1<br>-  | very small + indistinct<br>indistinct plaques<br>very small +<br>indistinct                                |

EXHIBIT

A6

(7/31) plaques

(6/3) marked in green.

## Counts of (5/25) Plaque Assays

| Sample                                 | Dilution                                    | A                               | B                              | C                              | Comments  |
|--|---|---------------------------------|--------------------------------|--------------------------------|---|
| (10) dl 738 (38-11)<br>(V5171)(710118) | $.5 \times 10^{-9}$<br>$.5 \times 10^{-10}$ | $\times 6$ 4<br>0 0             | $\times 1$ 11<br>0 0           | —<br>0 0                       | not too small, but<br>not very distinct<br>small & indistinct       |
| (11) 700/m41<br>(ASX920214)            | $.5 \times 10^{-9}$<br>$.5 \times 10^{-10}$ | $\times 7$ 8<br>$\times 0$ 2    | $\times 3$ 11<br>$\times 2$ 0  | $\times 12$ 10<br>$\times 1$ 2 | ~ like rec 700<br>quite large &<br>very distinct                    |
| (12) pm 734/m41 (AS)<br>(920402)       | $.5 \times 10^{-9}$<br>$.5 \times 10^{-10}$ | $\times 55$ 28<br>$\times 11$ 3 | $\times 13$ 31<br>$\times 6$ 4 | $\times 46$ 36<br>$\times 7$ 1 | ~ like rec 700<br>~ like rec 700                                    |
| (13) dl 716 (plg4)<br>(V5125)(700202)  | $.5 \times 10^{-9}$<br>$.5 \times 10^{-10}$ | $\times 26$ 62<br>$\times 1$ 5  | —<br>$\times 1$ 3              | —<br>—                         | quite large &<br>distinct<br>larger & more distinct<br>than rec 700 |
| (14) dl 753<br>(V578)(880624)          | $.5 \times 10^{-9}$<br>$.5 \times 10^{-10}$ | $\times 15$ 42<br>$\times 2$ 2  | —<br>$\times 0$ 3              | —<br>—                         | quite large &<br>distinct<br>very large & distinct                  |
| (15) mock                              | —   | 0 0                             | —                              | —                              | good monolayer  |



EXHIBIT

A7

## Counts of (7/25) Plaque Assays

| Sample                                      | Dilution                                    | A         | B         | C        | Comments  |
|---|---|-----------|-----------|----------|---|
| ① rec 700<br>(V5188)(910503)                | $.5 \times 10^{-9}$<br>$.5 \times 10^{-10}$ | +13<br>+0 | +16<br>+3 | -<br>+1  | plaques quite large & distinct, new plaques distinct                      |
| ② dl 742 (860313)                           | $.5 \times 10^{-9}$<br>$.5 \times 10^{-10}$ | +6<br>0   | +5<br>+1  | -<br>0   | new plaques very indistinct, older plaques still small with diffuse edges |
| ③ dl 745 (860313)                           | $.5 \times 10^{-9}$<br>$.5 \times 10^{-10}$ | +7<br>+2  | +7<br>+0  | -<br>+1  | large, distinct plaques; new plaques very distinct                        |
| ④ dl 712 (plg1)<br>(V5204)(911101)          | $.5 \times 10^{-9}$<br>$.5 \times 10^{-10}$ | +6<br>+0  | +10<br>0  | -<br>0   | small & indistinct older plqs still small                                 |
| ⑤ dl 730 (39-1)<br>(V5168)(901214)          | $.5 \times 10^{-9}$<br>$.5 \times 10^{-10}$ | +6<br>+1  | +8<br>+1  | -<br>+2  | very large & very distinct plaque new plqs distinct                       |
| ⑥ dl 732 ( $\frac{29}{1}$ )<br>(A5)(890414) | $.5 \times 10^{-9}$<br>$.5 \times 10^{-10}$ | +4<br>+0  | +2<br>+4  | -<br>+1  | very large & distinct plaque  |
| ⑦ pm 734 ( $\frac{19}{2}$ )<br>(890504)     | $.5 \times 10^{-9}$<br>$.5 \times 10^{-10}$ | +19<br>+2 | +17<br>+1 | +13<br>- | plaques a bit smaller than rec 700; new plaque quite distinct             |
| ⑧ dl 735 ( $\frac{34}{1}$ )<br>(890425)     | $.5 \times 10^{-9}$<br>$.5 \times 10^{-10}$ | +6<br>+1  | +12<br>+1 | +13<br>- | small & very indistinct plaq  |
| ⑨ dl 736 ( $\frac{0.04}{1}$ )<br>(890414)   | $.5 \times 10^{-9}$<br>$.5 \times 10^{-10}$ | +5<br>+1  | +5<br>0   | +6<br>-  | very small & very indistinct (both old & new)                             |

(4/5) max (2.4) m B

## EXHIBIT

A8

## Counts of (5/25) plaque Assays.

| Sample                                 | Dilution                                    | A         | B         | C         | Comments  |
|--|---|-----------|-----------|-----------|---|
| (10) dl 738 (38-11)<br>(V5171)(910118) | $.5 \times 10^{-9}$<br>$.5 \times 10^{-10}$ | +5<br>+1  | +6<br>0   | —<br>0    | new plaques very small & indistinct, even older plaques are ~ 0 size with diffuse edges       |
| (11) 700/m41<br>(A5)(920214)           | $.5 \times 10^{-9}$<br>$.5 \times 10^{-10}$ | +5<br>+0  | +2<br>+0  | +5<br>+0  | plaques large, new plaques very distinct (similar size to much older de 738)                  |
| (12) pm 734/m49(A5)<br>(920402)        | $.5 \times 10^{-9}$<br>$.5 \times 10^{-10}$ | +17<br>+4 | +16<br>+1 | +17<br>+5 | new plaques very distinct & fairly large & distinct, maybe a bit smaller than rec 700 plaques |
| (13) dl 716 (plg 4)<br>(V5125)(900202) | $.5 \times 10^{-9}$<br>$.5 \times 10^{-10}$ | +1<br>+1  | —<br>+1   | —<br>—    | very large & distinct (exp. on $10^{-10}$ )   |
| (14) dl 753<br>(V578)(880624)          | $.5 \times 10^{-9}$<br>$.5 \times 10^{-10}$ | +7<br>+1  | —<br>+2   | —<br>—    | very large & distinct   |
| (15) mock                              | —   | 0         | —         | —         | good monolayer  |

EXHIBIT

A9

## Counts of (Fcs) Plaque Assays

| Sample                              | Dilution                                    | A               | B               | C        | Comments   |
|-------------------------------------|---|-----------------|-----------------|----------|--|
| ① rec 700<br>(V5138)(910503)        | $.5 \times 10^{-9}$<br>$.5 \times 10^{-10}$ | +3<br>+1        | +6<br>+1        | -<br>+1  | distinct fairly<br>large plaques                             |
| ② dl 742 (860313)                   | $.5 \times 10^{-9}$<br>$.5 \times 10^{-10}$ | +20<br>+3       | +22<br>+2       | -<br>+1  | small & indistin.<br>plaques; new<br>plaques "pinpoint"      |
| ③ dl 745 (860313)                   | $.5 \times 10^{-9}$<br>$.5 \times 10^{-10}$ | +7<br>+0        | +2<br>+0        | -<br>+1  | large distinct<br>plaques                                    |
| ④ dl 712 (ply 1)<br>(V5204)(911101) | $.5 \times 10^{-9}$<br>$.5 \times 10^{-10}$ | +28<br>+1       | +29<br>+1       | -<br>+2  | very small plga;<br>new plga "pinpoint"                      |
| ⑤ dl 730 (39-4)<br>(V5168)(901214)  | $.5 \times 10^{-9}$<br>$.5 \times 10^{-10}$ | +1<br>+1 (mild) | +4<br>+0        | -<br>+0  | very large, nice<br>plaques<br>(edges a bit diffuse)         |
| ⑥ dl 732 (2%)<br>(A5)(890414)       | $.5 \times 10^{-9}$<br>$.5 \times 10^{-10}$ | +7<br>+0        | +1<br>+0 (mild) | -<br>+1  | very large, nice<br>plaques                                  |
| ⑦ pm 734 (1 1/2)<br>(890504)        | $.5 \times 10^{-9}$<br>$.5 \times 10^{-10}$ | +28<br>+2       | +28<br>+3       | +25<br>- | slightly small<br>plaques, new<br>plaques fairly<br>distinct |
| ⑧ dl 735 (3 1/1)<br>(890425)        | $.5 \times 10^{-9}$<br>$.5 \times 10^{-10}$ | +18<br>+5       | +34<br>+7       | +30<br>- | older plga times<br>new plga ~<br>"pinpoints"                |
| ⑨ dl 736 (0.04%)<br>(890414)        | $.5 \times 10^{-9}$<br>$.5 \times 10^{-10}$ | +11<br>+0       | +22<br>+0       | +15<br>- | very small plga<br>new plga<br>"pinpoints"                   |

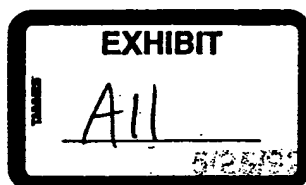
EXHIBIT

A10

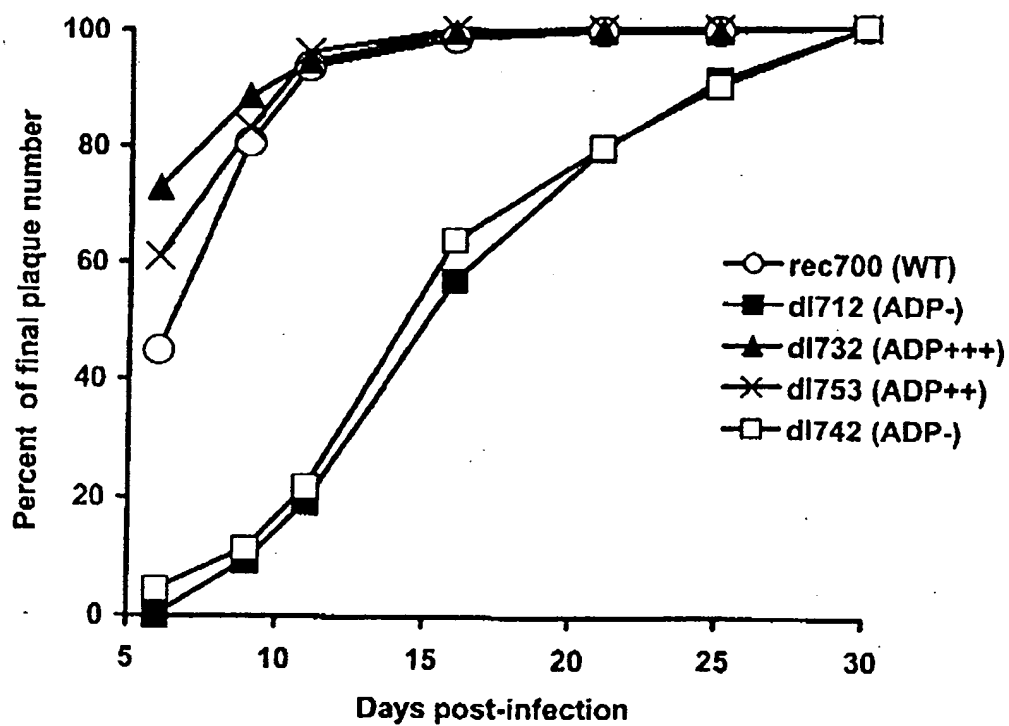
(18) marked in black

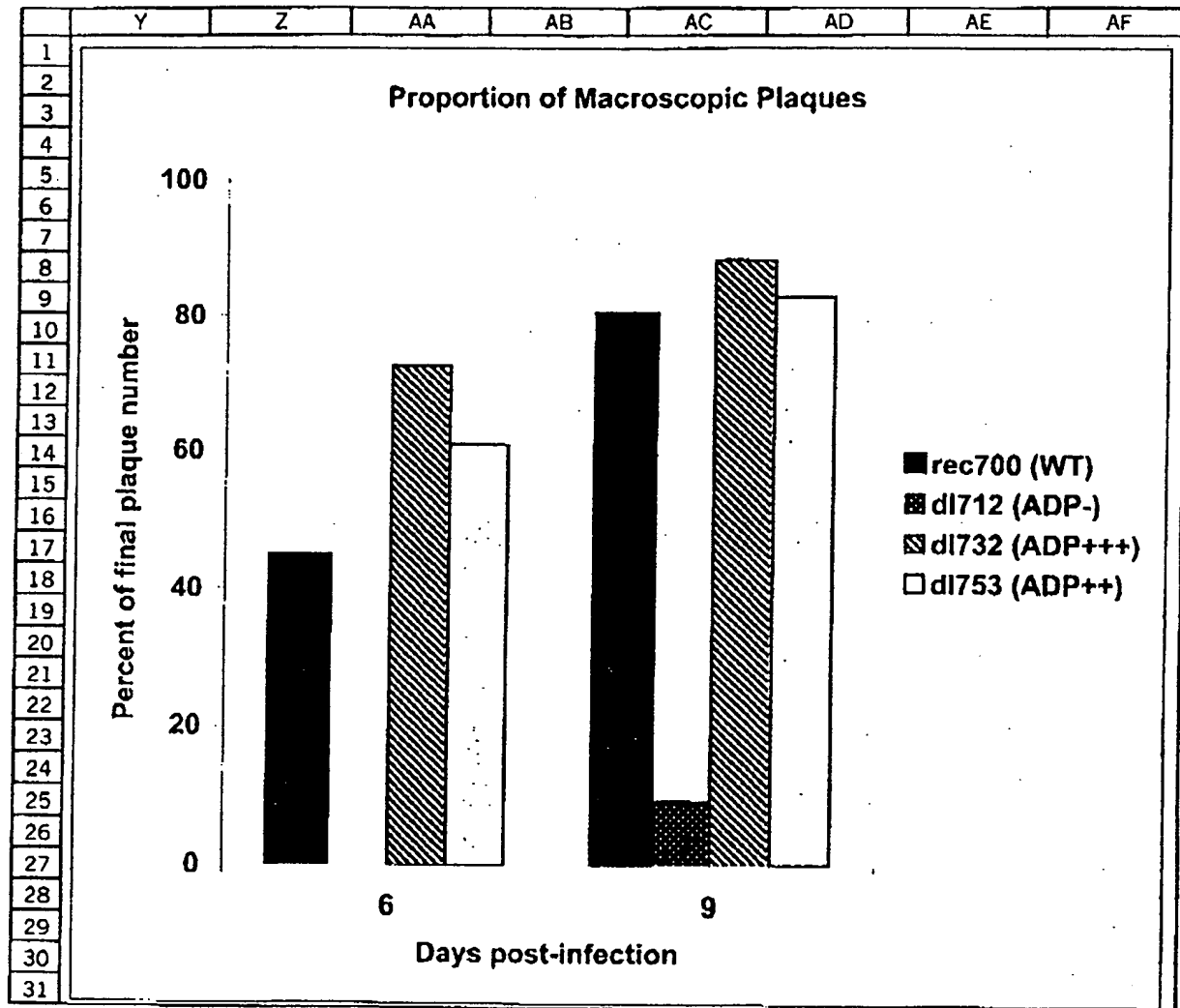
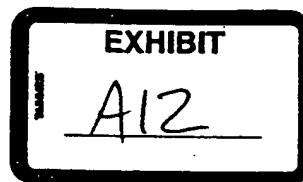
## Counts of (5/25) plaque Assays

| Sample                                 | Dilution                                    | A         | B         | C         | Comments  |
|--|---|-----------|-----------|-----------|---|
| (10) dl 738 (38-11)<br>(VS171)(910118) | $.5 \times 10^{-9}$<br>$.5 \times 10^{-10}$ | +22<br>+2 | +16<br>+3 | -<br>+3   | small &<br>indistinct, new<br>plaques very small                            |
| (11) 700/m41<br>(AS)(920214)           | $.5 \times 10^{-9}$<br>$.5 \times 10^{-10}$ | +3<br>+1  | +5<br>+0  | +4<br>+0  | new plaques larger than<br>oldest of dl 738;<br>large plaques<br>& distinct |
| (12) pm 734/m49(AS)<br>(920402)        | $.5 \times 10^{-9}$<br>$.5 \times 10^{-10}$ | +5<br>+2  | +7<br>+3  | +15<br>+4 | large distinct<br>plaques (new plaques<br>larger than oldest (738))         |
| (13) dl 716 (ply 4)<br>(VS125)(900202) | $.5 \times 10^{-9}$<br>$.5 \times 10^{-10}$ | +3<br>+1  | -<br>+1   | -<br>-    | very large &<br>distinct  |
| (14) dl 753<br>(VS 78)(880624)         | $.5 \times 10^{-9}$<br>$.5 \times 10^{-10}$ | +1<br>+0  | -<br>+2   | -<br>-    | very large &<br>distinct  |
| (15) mock                              | -   | 0         | -         | -         | good monolayer  |



plaque assay

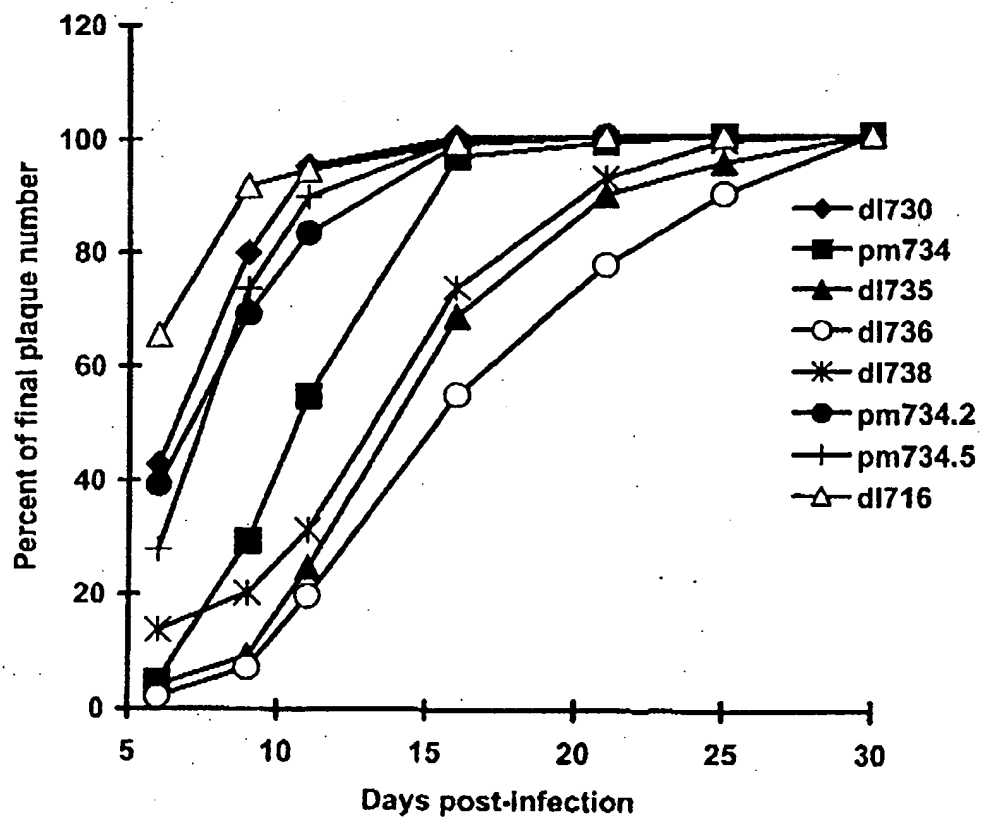




**EXHIBIT**

A13

0125802 plaque assay

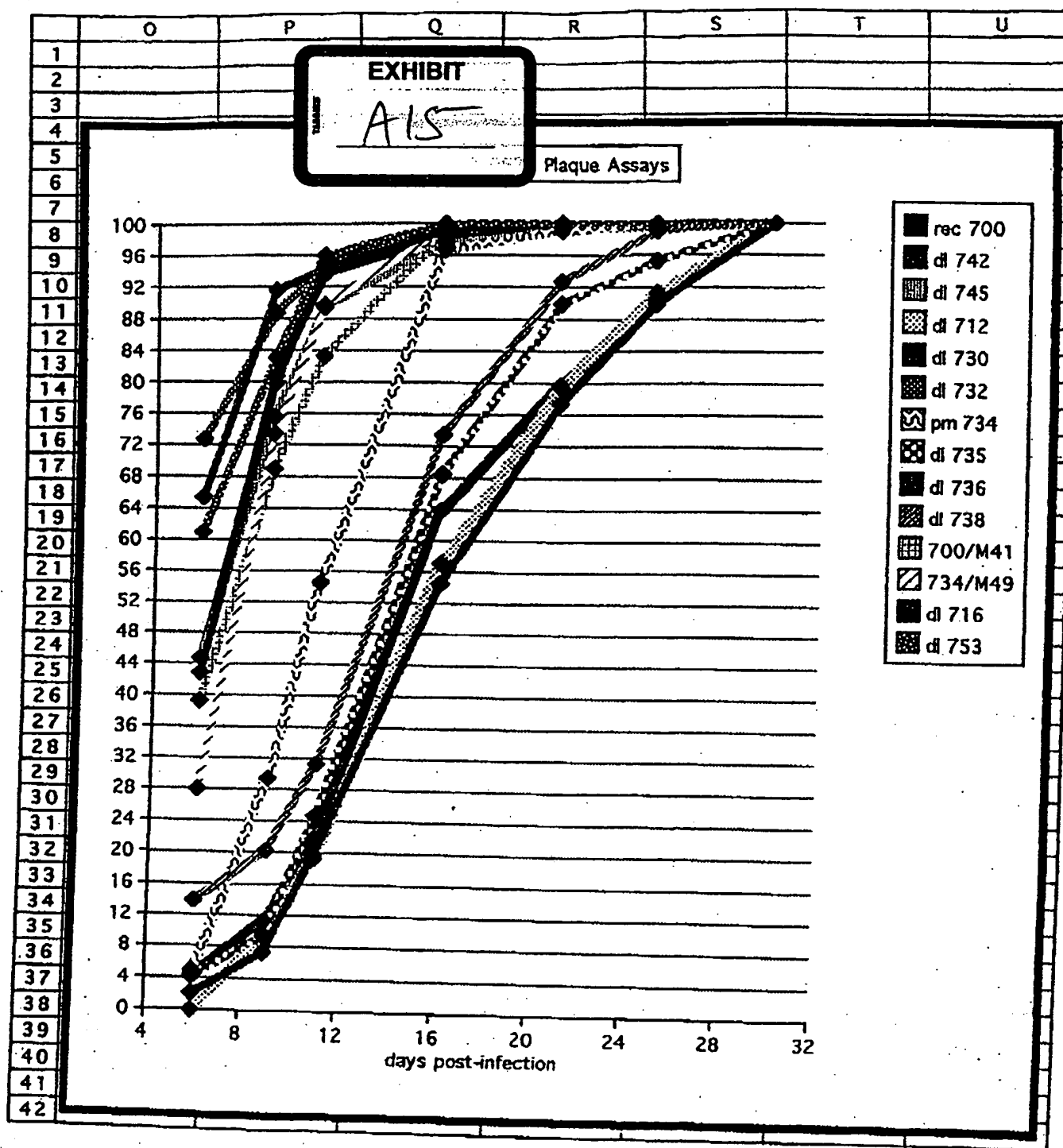


## EXHIBIT

A14

|    | B              | C    | D    | E    | F    | G    | H   |
|----|----------------|------|------|------|------|------|-----|
| 2  | 6              | 9    | 11   | 16   | 21   | 25   | 30  |
| 3  | rec700 (WT)    | 44.8 | 80.6 | 93.7 | 98.4 | 99.6 | 100 |
| 4  | dl712 (ADP-)   | 0    | 9.3  | 19.3 | 57.1 | 79.5 | 100 |
| 5  | dl732 (ADP+++) | 72.8 | 88.6 | 94.6 | 99.5 | 99.5 | 100 |
| 6  | dl753 (ADP++)  | 61   | 83.1 | 96.1 | 100  | 100  | 100 |
| 7  | dl742          | 4.4  | 11.4 | 21.9 | 64   | 79.8 | 100 |
| 8  | dl745          | 44.7 | 75.6 | 89.4 | 97.6 | 99.2 | 100 |
| 9  |                | 6    | 9    | 11   | 16   | 21   | 30  |
| 10 | dl730          | 42.9 | 79.8 | 95.1 | 100  | 100  | 100 |
| 11 | pm734          | 4.9  | 29.3 | 54.6 | 96.6 | 99   | 100 |
| 12 | dl735          | 4.2  | 9.3  | 24.7 | 68.4 | 89.8 | 100 |
| 13 | dl736          | 2.2  | 7.3  | 19.7 | 54.7 | 77.4 | 100 |
| 14 | dl738          | 13.8 | 20.2 | 31.2 | 73.4 | 92.7 | 100 |
| 15 | pm734.2        | 39.3 | 69   | 83.3 | 98.8 | 100  | 100 |
| 16 | pm734.5        | 27.9 | 73.4 | 89.7 | 99.5 | 100  | 100 |
| 17 | dl716          | 65.4 | 91.6 | 94.4 | 99.1 | 100  | 100 |
| 18 |                |      |      |      |      |      |     |
| 19 |                |      |      |      |      |      |     |
| 20 |                |      |      |      |      |      |     |
| 21 |                |      |      |      |      |      |     |
| 22 |                |      |      |      |      |      |     |
| 23 |                |      |      |      |      |      |     |
| 24 |                |      |      |      |      |      |     |
| 25 |                |      |      |      |      |      |     |
| 26 |                |      |      |      |      |      |     |
| 27 |                |      |      |      |      |      |     |
| 28 |                |      |      |      |      |      |     |
| 29 |                |      |      |      |      |      |     |
| 30 |                |      |      |      |      |      |     |
| 31 |                |      |      |      |      |      |     |





A16

[illegible]

FAX NO. :314

P.A. (p. 2)

[illegible]

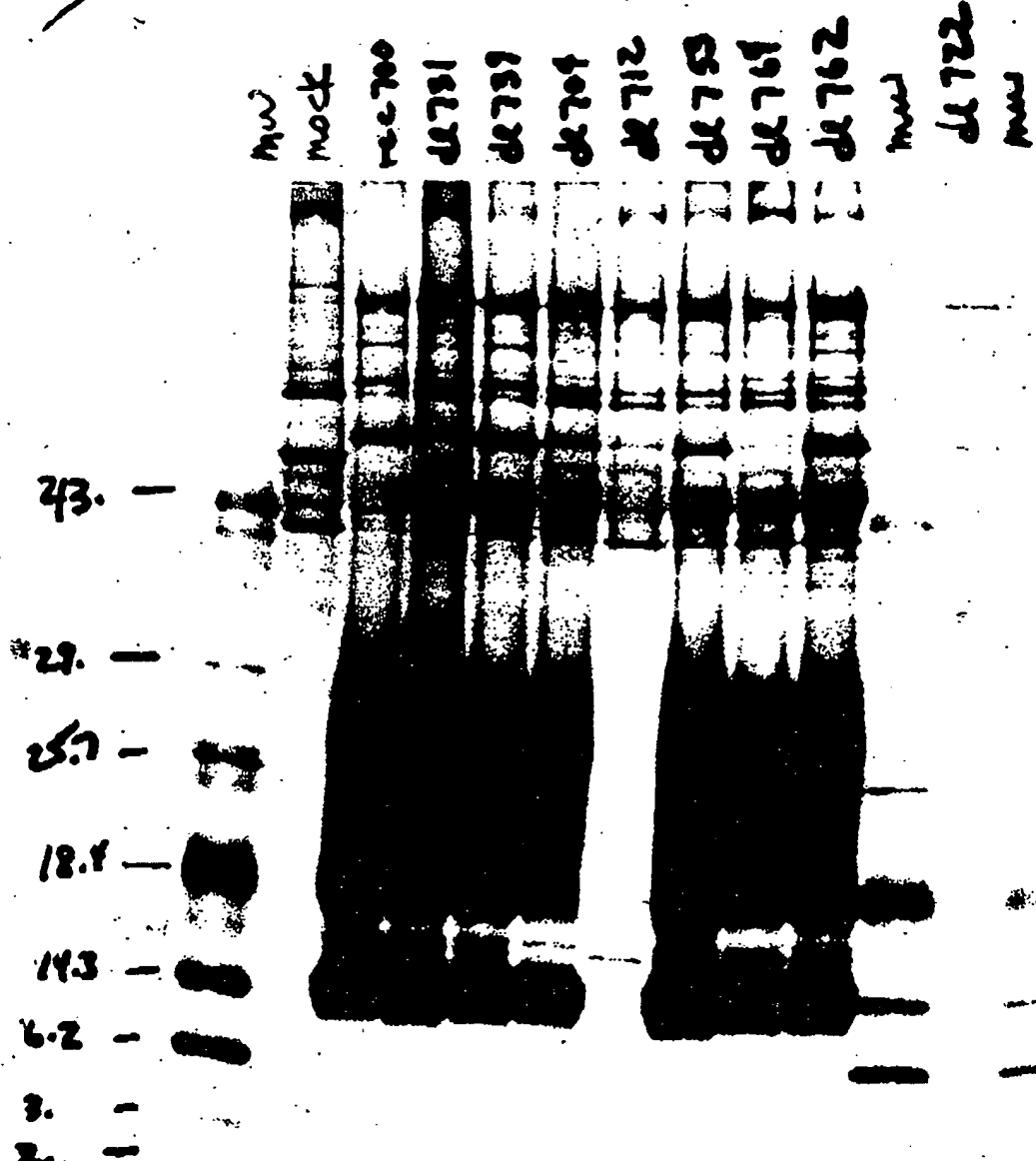


EXHIBIT  
A18

Gel #1

13 day exposure  
5 min developer

709 #1

11.6 (Deletion Mutation)

8/9 →

43. —

29. —

25.7 —

18.1 —

14.3 —

6.2 —

3. —

2. —

EXHIBIT

A19

Gal #1

13 day exposure

5 min developer

709 #1

11.6 (Petition letter)

8/9 →

EXHIBIT

FROM : SAINT LOUIS UNIVE

A20

:3147733403

Dec. 19 2002 10:39AM P4

7 day exposure (5 min developer)

916 780  
730  
745  
732  
742  
735  
736  
737  
738  
Ad2



A21

Stained by A.S.

| set #1 |                          | set #2 |                            | set #3 |                           |
|--------|--------------------------|--------|----------------------------|--------|---------------------------|
| 1      | mock $\alpha 11.6(2-16)$ | 13     | 714 $\alpha 11.6(2-16)$    | 25     | 732 $\alpha 11.6(87-101)$ |
| 2      | rec 700 $\alpha$ Fiber   | 14     | 715 $\alpha$ Fiber         | 26     | 742                       |
| 3      | dl 712                   | 15     | 713                        | 27     | 735                       |
| 4      | 730                      | 16     | 25-mer                     | 28     | 737                       |
| 5      | 745                      | 17     | 734                        | 29     | 738                       |
| 6      | 732                      | 18     | 734/MS6                    | 30     | 35-mer                    |
| 7      | 742                      | 19     | 734/MS9                    | 31     | 714                       |
| 8      | 735                      | 20     | mock $\alpha 11.6(87-101)$ | 32     | 715                       |
| 9      | 736                      | 21     | rec 700                    | 33     | 25-mer                    |
| 10     | 737                      | 22     | dl 712                     | 34     | 734                       |
| 11     | 738                      | 23     | 730                        | 35     | 734/56                    |
| 12     | 35-mer                   | 24     | 745                        | 36     | 734/49                    |

1st Ab's:

 $\alpha 11.6(2-16)$  (910830 preads 2X; 1:4 dil)

#98689 bleed out

$$800 \mu\text{l} \left[ \begin{array}{l} (2-16) (1:200) \leftarrow 16 \mu\text{l} \\ \text{Fiber} (1:500) \leftarrow 1.6 \mu\text{l} \leftarrow \text{mt} \end{array} \right.$$
 $\alpha 11.6(87-101)$  (910830 preads 4X; 1:4 dil)

#98686 bleed out

$$720 \mu\text{l} \left[ (87-101) (1:400) \right. \quad \frac{7.2 \mu\text{l}}{712.8}$$

2nd Ab's:

(all 1:50 dil's)

[ $\alpha$  Rabbit-FITC /  $\alpha$  Mouse-RITC]or  $\alpha$  Rabbit-FITC

A22

slides)

920730

## Descriptions of AS 11.6K Mutants (IF)

- ① - ~~10~~  $\alpha$ 11.6K (Z-16) (1:200 dil) (1:400  $\alpha$ Fiber-RITC)
- ① L-mock - ~no background for fiber; slight background for  $\alpha$ 11.6K (esp. for mitotic spindle poles)  
R-rec 700 - all in late stage (by fiber staining); ~20-30% of cells stain for 11.6K (n.c. & Golgi primarily), some "debris" or aggregated material
- ② L-dl 712 - 100% inf'n; no 11.6K staining  
R-dl 730 - 20-30% stain for 11.6K  $\rightarrow$  maybe more Golgi & debris staining; 100% infected
- ③ L-dl 745 - ~100% inf'n; poss. very diffuse Golgi staining  $\rightarrow$  not very bright & see no n.c. staining  
R-dl 732 - 70-80% stain for 11.6K  $\rightarrow$  n.c., Golgi (Golgi staining reduced); a lot of aggregated material
- ④ L-dl 742 - looks ~like dl 712  
R-dl 735 - 100% inf'n; very little 11.6K staining (poss a bit in Golgi, not really n.c.)
- ⑤ L-dl 736 - 100% inf'n; little if any staining, poss some aggreg'd material but not definitive  
R-dl 737 - 100% inf'n; bright staining; 30-40% of cells stained  $\rightarrow$  n.c., debris & virus replication sites
- ⑥ L-dl 738 - 100% inf'n; 11.6K aggregated material  
R-35-mer - " " ; not so much n.c. staining & Golgi staining is very diffuse; more e.r.?
- ⑦ L-dl 714 - ~30% of cells stained, good n.c. & Golgi but also a lot of e.r. (do not see plasma membrane)  
R-dl 715 - like 714 except less n.c. staining & more aggregated material
- ⑧ L-pm 734 - like 712  
R-734/mSL - " " " " " " " "



EXHIBIT

A23

## Descriptions (cont'd)

- ⑨ L-dl 713- 50-60% stained,  $\alpha$  n.c., Golgi + a lot of e.r. maybe more aggregated material  
R- 25-mer - less n.c. staining; seemingly more Golgi staining; seemingly less staining overall
- ⑩ L-pm 734/m49 - like dl 712  
~~#11~~ - ⑪  $\alpha$  11.6K (87-101) (1:400 dil.)
- ⑪ L-mock - slight Golgi-like pattern (very light)  
R-rec 700 - ~100% stained, good n.c., Golgi; more e.r. + particulate than sometimes
- ⑫ L-dl 712 - a bit anti-nuclear (not 11.6K pattern)  
R-dl 730 - ~100% of cells; very bright, maybe a bit more into all membranes (but n.c. + Golgi most)
- ⑬ L-dl 745 - more "grainy" staining + more aggregated material, otherwise quite "normal"  
R-dl 732 - almost 100% with very bright n.c. staining, also Golgi + some e.r. (brighter than rec 700)
- ⑭ L-dl 742 - like 712  
R-dl 735 - almost only Golgi (very reduced n.c.)
- ⑮ R-dl 737 - very bright; typical pattern but more particulate + more e.r.
- ⑯ L-dl 738 - not in membranes; some diffuse staining but mainly lots of aggregated material  
R-35-mer - bright; in all membranes
- ⑰ L-dl 714 - very bright; in all membranes  
R-dl 715 - less n.c. and more in plasma membrane (than dl 714)
- ⑱ R-25-mer - more Golgi, less n.c., vesicles or particles in cytoplasm
- ⑲ L-pm 734 - fairly typical but "grainier" appearance  
R-734/m56 - sim to p 734 but not quite as bright
- ⑳ L-734/m49  $\rightarrow$  sim to 734, maybe more Golgi + associated st.

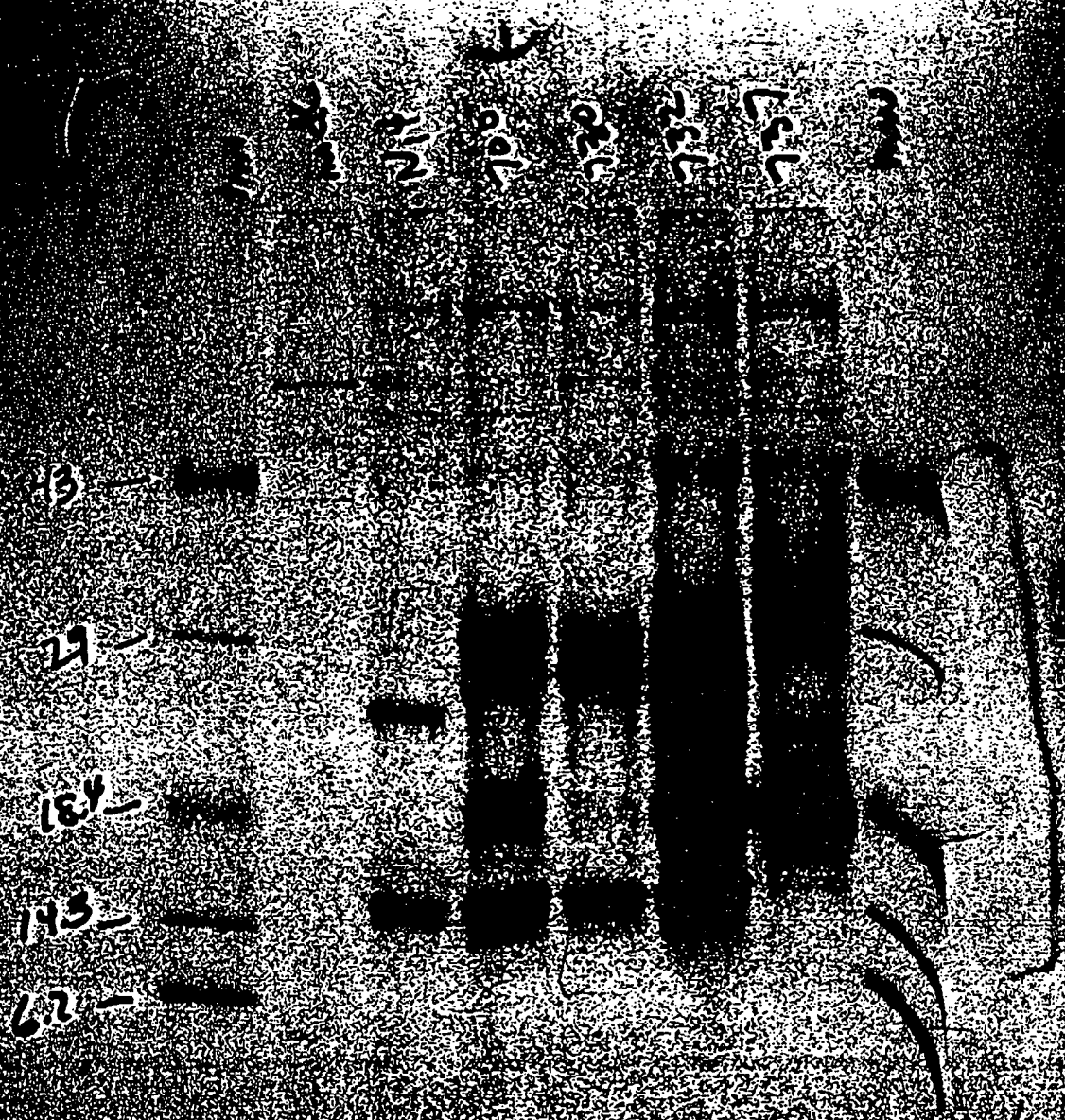
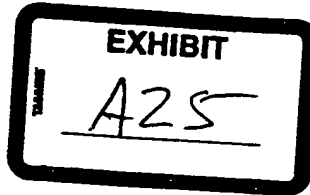
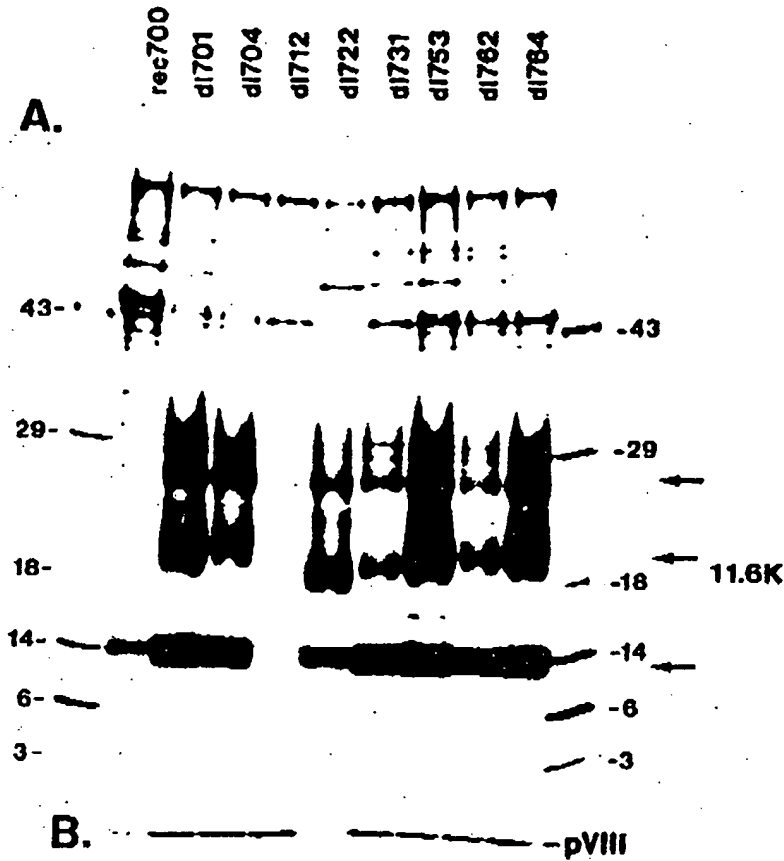


EXHIBIT  
1 A24

#1 100% 4 day exposure



#  
IF  
9108271  
pnb wt?



Have all these  
prints.

Fig. 1

900331 gel #1  
~ 11.6K (87-101)  
like ext. cell

(43) #1 ← (43) IF 11.6K

A26

(checking for 11.01.70)

## Assays of Virus Release from A549 cells

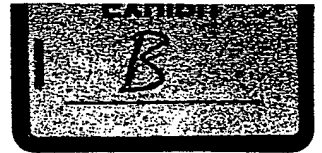
| Sample # | Infection | Sample Plated cells | pfu    | Time (p.i.) | PA date   | PA dilution (all 5#)        |
|----------|-----------|---------------------|--------|-------------|-----------|-----------------------------|
| ①        | mock      | $2 \times 10^6$     | —      | 7½h         | —         | —                           |
| ②        | mock      | $5 \times 10^5$     | —      |             | —         | —                           |
| ③        | rec 700   | $2 \times 10^6$     | $10^5$ |             | (4/28) AT | $5 \times 10^0, 10^{-1}$    |
| ④        | ↓         | $5 \times 10^5$     | $10^5$ |             |           | $10^0, 10^{-1}$             |
| ⑤        | ↓         | $2 \times 10^6$     | $10^3$ |             |           | $10^0$                      |
| ⑥        | de 712    | $2 \times 10^6$     | $10^5$ |             |           | $10^0, 10^{-1}$             |
| ⑦        | ↓         | $5 \times 10^5$     | $10^5$ |             |           | $10^0, 10^{-1}$             |
| ⑧        | ↓         | $2 \times 10^6$     | $10^3$ |             |           | $10^0$                      |
| ⑨        | ↓         | $5 \times 10^5$     | $10^3$ |             |           | $10^0$                      |
| ⑩        | de 753    | $2 \times 10^6$     | $10^5$ |             |           | $10^0, 10^{-1}$             |
| ⑪        | ↓         | $5 \times 10^5$     | $10^5$ |             |           | $10^0, 10^{-1}$             |
| ⑫        | ↓         | $2 \times 10^6$     | $10^3$ |             |           | $10^0$                      |
| ⑬        | mock      | $2 \times 10^6$     | —      | 24h         | (6/29) AT | $10^0$                      |
| ⑭        | mock      | $5 \times 10^5$     | —      |             |           | $10^0$                      |
| ⑮        | rec 700   | $2 \times 10^6$     | $10^5$ |             |           | $10^{-1}, 10^{-3}, 10^{-5}$ |
| ⑯        | ↓         | $5 \times 10^5$     | $10^5$ |             |           | $10^{-1}, 10^{-3}, 10^{-5}$ |
| ⑰        | ↓         | $2 \times 10^6$     | $10^3$ |             |           | $10^0, 10^{-2}, 10^{-4}$    |
| ⑱        | de 712    | $2 \times 10^6$     | $10^5$ |             |           | $10^{-1}, 10^{-3}, 10^{-5}$ |
| ⑲        | ↓         | $5 \times 10^5$     | $10^5$ |             |           | $10^{-1}, 10^{-3}, 10^{-5}$ |
| ⑳        | ↓         | $2 \times 10^6$     | $10^3$ |             |           | $10^0, 10^{-2}, 10^{-4}$    |
| \(21\)   | ↓         | $5 \times 10^5$     | $10^3$ |             |           | $10^0, 10^{-2}, 10^{-4}$    |
| \(22\)   | de 753    | $2 \times 10^6$     | $10^5$ |             |           | $10^{-1}, 10^{-3}, 10^{-5}$ |
| \(23\)   | ↓         | $5 \times 10^5$     | $10^5$ |             |           | $10^{-1}, 10^{-3}, 10^{-5}$ |
| \(24\)   | ↓         | $2 \times 10^6$     | $10^3$ |             |           | $10^0, 10^{-2}, 10^{-4}$    |

at indicated times p.i., 1 ml of sup was removed, cells were pelleted (microfuge, 3 min) + sup removed to new Eppendorf; PA's of 0.5 ml volumes (single dishes); added back 1 ml of <sup>fresh</sup> DME (2% FCS) to each dish to replace volume removed

## **EXHIBIT B**



SAINT LOUIS UNIVERSITY  
HEALTH SCIENCES CENTER



SCHOOL OF MEDICINE

1402 S. Grand Blvd.  
St. Louis, MO 63104  
314/577-8435  
FAX 314/773-3403

Department of Molecular  
Microbiology & Immunology

William S. M. Wold, Ph.D.  
Professor and Chairman

Dr. Rae Lyn Burke  
Chiron Corporation  
Department of Virology  
4560 Horton Street  
Emeryville, CA 94608

Dear Rae Lyn,

I have finally returned to my office and cleared off the stack of work on my desk. Thank you for providing the names of venture capitalists, and for contacting Rajen Dalal. I have decided to take the less visionary approach mentioned in your letter of June 10, 1994, and to request funding from Chiron for the gene therapy project on the adenovirus E3-11.6K protein that promotes cell death. The proposal is enclosed. I have submitted a patent application for this project; if the patent is awarded, it would provide protection in the U.S.A. but not in Europe or Asia.

Also enclosed is a budget for the proposal. I anticipate carrying out the research in my laboratory here at St. Louis University. However, other alternatives, including doing the work at Chiron, are also possible.

I have requested a consultant fee for myself and Dr. Ann Tollefson. Ann is an Associate Research Professor working in my laboratory; she is the co-discoverer of the cell death-promoting properties of 11.6K. She will co-direct the project and she will perform benchwork on the project. Note that our consultant fees are separate from the requested budget for the project.

I am also considering establishing a corporation, perhaps in August, with St. Louis University as a minor partner. If Chiron decides to fund this project, perhaps the funding can be channeled through this corporation. Therefore, the budget for the project should be considered to be tentative.

Our Biotechnology Transfer Office requires that an appropriate official from Chiron sign a confidentiality agreement. The form is enclosed.

Dr. Rae Lyn Burke

page 2

Thank you again for your interest, and for your hospitality during my visit.

With best regards,

A handwritten signature in cursive script, appearing to read "Bill".

William S. M. Wold, Ph.D.  
Professor and Chairman

WSMW:jlw

enclosures

## BUDGET

|  |          |
|--|----------|
| <u>Personnel:</u>                            |          |
| Lynda K. Hawkins, Ph.D.                      | \$20,700 |
| TBA Postdoctoral Fellow                      | 25,600   |
| Fringes                                      | 9,955    |
| Subtotal Personnel                           | 56,255   |
| <br><u>Supplies:</u>                         |          |
|  | 24,000   |
| <br><u>Other:</u>                            |          |
| Maintenance contracts and repair             | 1,200    |
| Telephone, postage, photocopier<br>demurrage | 400      |
| Radiation disposal                           | 500      |
| Publication, illustrations                   | 500      |
| Subtotal other expenses                      | 2,600    |
| Subtotal                                     | 82,855   |
| Indirect costs (10%)                         | 8,286    |
| TOTAL  | \$91,141 |
| <br>Consultant costs*                        |          |
|  | \$12,000 |

\*In addition to total project budget.



CONFIDENTIALITY AGREEMENT

THIS AGREEMENT is made by and between St. Louis University Medical Center having a principle place of business located at 1402 South Grand Blvd., St. Louis, MO 63140 and Chiron Corporation a corporation having a principle place of business located.....

THE PARTIES HAVE AGREED AND DO AGREE AS FOLLOWS:

1. This AGREEMENT refers to the work of Dr.....  
William S. M. Wold.....  
relating to adenovirus 11.6K protein that functions in  
promoting cell death.....
2. .... agrees to maintain the confidentiality of Proprietary Information discussed during their meetings. Proprietary Information shall not either be used or disclosed to any other person or entity without the written consent of the St. Louis University Medical Center except as may be required under a court order.
3. The confidentiality obligation shall not apply to:
  - a: Information which ..... can establish by reasonable proof was in its possession at the time of disclosure.
  - b: Public domain information or information which after disclosure becomes part of the public domain information by publication or otherwise except by breach of this Agreement.

The term and condition herein are acceptable to both parties of this Agreement as evidenced by the signatures of their authorized representatives:

Signature\_\_\_\_\_

Date: \_\_\_\_\_

Name: Aftab Alam

Title: Director, Biotechnology Transfer Research Center

Organization: St. Louis University Medical Center

Signature\_\_\_\_\_

Date

Name/Print\_\_\_\_\_Title:\_\_\_\_\_

Organization:.....

## ADENOVIRUS E3-11.6K PROTEIN AS A CELL DEATH-PROMOTING AGENT

### A. BACKGROUND

The human adenovirus type 2 (Ad2) and Ad5 E3-11.6K protein (MW of 11,600) (Fig. 1) was first identified by our laboratory, using an antipeptide antiserum to immunoprecipitate the protein from adenovirus-infected cells (Wold et al., 1984). Subsequently, we have prepared three additional antipeptide antisera, we have isolated mutants that completely delete the gene, and we have constructed a series of about 20 mutants with truncations, small in-frame deletions, and missense mutations in the gene (Fig. 2; Tollefson et al., 1992; unpublished results). Using these reagents, we have shown that 11.6K is synthesized in low amounts during early stages of infection (prior to viral DNA replication), but in large amounts at late stages of infection (Tollefson et al., 1992). We have also shown that 11.6K is an N-linked O-linked integral membrane protein that initially localizes to the endoplasmic reticulum (ER) and Golgi apparatus, but ultimately localizes to the nuclear membrane (Scaria et al., 1992).

We have recently discovered that the 11.6K protein functions to promote cell death (A.E. Tollefson, A. Scaria, and W.S.M. Wold, manuscript in preparation). A representative experiment is shown in Fig. 3. As measured by release of lactate dehydrogenase (LDH), cells infected with wild-type adenovirus (*rec700*) begin to die at 2-3 days postinfection (p.i.), and are mostly dead by 5-7 days p.i. In contrast, cells infected with a mutant (*d1712*) that deletes only the 11.6K gene stay completely alive until 5 days postinfection, and they do not begin to die until 6 days p.i. Similar results have been obtained using, as indicators of cell death, the release of adenovirus from infected cells, trypan blue exclusion, the MTT assay (which measures mitochondrial activity), DNA degradation (agarose gels, DAPI staining, Apo-Tag), and light and electron microscopy. We believe that the function of 11.6K in adenovirus biology is to lyse

cells, thereby allowing adenovirus to be released from the infected cell.

Using our collection of virus mutants in the 11.6K gene, we are currently performing a structure-function analysis of 11.6K. Some of the mutants are shown in Fig. 2. The phenotypes of the mutants are summarized at the right side of Fig. 2. SDS-PAGE analysis indicated that all the mutant proteins are stable with the possible exception of *pm734.1* which only synthesizes residues 49-101 of the 11.6K protein (data not shown). These mutants have allowed us to map the protein domains required to target 11.6K to the nuclear membrane, and for 11.6K to promote cell death. Briefly, residues 41-59, the only hydrophobic domain in the protein, serves as the signal to insert 11.6K into membranes and to anchor 11.6K within membranes, i.e., this domain is a signal-anchor sequence. Residues 63-78, which includes the basic-proline domain (residues 63-74), a domain rich in basic amino acids and proline, is required to target 11.6K *specifically* to the nuclear membrane; mutant proteins that lack these sequences localize to *all membranes*, not specifically to the nuclear membrane. Regarding cell death, residues 1-40 and 71-101 can be deleted without abrogating the ability of 11.6K to promote cell death. Thus, the "death domain" appears to consist of residues ca. 46-60, with help from residues 61-74. However, we emphasize that although we have shown that these sequences are necessary to promote cell death, we have not shown that they are sufficient.

We do not understand the mechanism of action of 11.6K in targeting to the nuclear membrane and in promoting cell death. We believe that the nuclear membrane is the site of action of 11.6K because certain mutant proteins that do not exit from the ER or Golgi are defective in promoting cell death. Future studies in the laboratory will focus on the mechanism of action of 11.6K. This mechanism is not only of fundamental interest, but it may also

elucidate the cellular mechanisms that control cell death; this latter information may allow for novel gene therapy approaches for killing or protecting cells.

## B. PROPOSAL

Since the 11.6K protein can promote the death of adenovirus-infected cells, it has the potential use as a therapeutic agent to kill cells, e.g. malignant cells, in humans. This proposed research addresses two issues that must be resolved in order for 11.6K to be used as a therapeutic agent.

First, although we know that 11.6K promotes the death of *adenovirus-infected cells*, we do not know whether 11.6K can *function autonomously* to kill cells.

Second, since 11.6K functions inside the cell, apparently at the nuclear membrane, a means must be developed to deliver the protein to the cells of interest.

## C. EXPERIMENTAL PROCEDURES

### C.1. Can the 11.6K protein function autonomously to kill cells?

In order to answer this question, we have attempted to isolate human A549 cells stably transfected with the 11.6K gene. Several neo<sup>R</sup>-resistant cell lines have been obtained. These cells express only low levels of 11.6K, and the 11.6K protein is localized in the Golgi but not the nuclear membrane. Since we were not able to obtain cells that express high levels of 11.6K, or cells wherein 11.6K is localized to the nuclear membrane, these experiments suggest, but do not prove, that 11.6K can function autonomously to kill cells. We propose two approaches to address this question in more detail.

#### C.1.a. Tet-inducible vector.

We have obtained the *tet*-inducible vector system from Hermann Bujard

(Gossen and Bujard, 1992). In the *tet* system, the tetracycline repressor (tetR) was fused to the transactivation domain of VP16. In the absence of tetracycline, the fusion protein will bind to *tet* operators and will transactivate a promoter consisting of *tet* operators plus a TATA box. In the presence of tetracycline, the tetR-VP16 fusion protein does not bind to *tet* operators, and the promoter is silent. The major advantages of this system are that, upon removal of tetracycline, the gene is induced by up to five orders of magnitude. Also, induction is rapid, being >20% in 4 h and 100% in 12 h. Finally, the low amount (0.1  $\mu\text{g/ml}$ ) of tetracycline required to keep the gene silent is unlikely to affect other properties of the cell. We will clone 11.6K into the *tet* vector, develop cell lines, and examine whether 11.6K is induced by removal of tetracycline, and whether 11.6K promotes cell death following induction. Cell death will be measured by release of LDH, trypan blue exclusion, the MTT assay, and microscopy.

If 11.6K alone does promote cell death, then we will proceed with the experiments described in Section D below. If 11.6K alone does not promote cell death, then we will proceed with the experiments in Section C.3.

#### **C.1.b. Adenovirus vector.**

There are two general classes of adenovirus vectors, nondefective and defective for replication in cultured human cells. The nondefective vectors generally have the E3 transcription unit (Fig. 4) deleted and replaced with the transgene. The E3 genes are not required for virus replication in cultured cells or in the lungs of hamsters or cotton rats, so these vectors are able to replicate. However, the E3 genes function to block the immune and inflammatory response to virus infection, so the E3-deleted vectors are more pathogenic than wild-type adenovirus.

Defective vectors generally have the E1A and E1B regions deleted and replaced with the transgene, and they have the E3 region deleted in order to increase the amount of foreign DNA that can be inserted into the viral genome (only 105% of the genome can be packaged). The E1A and E1B genes (E1B-55K) are essential for virus replication. E1A proteins are required to efficiently induce transcription of the other adenovirus transcription units. The E1B-55K protein is required for efficient transport of viral "late" mRNAs from the nucleus to the cytoplasm. Since the defective vectors lack E1A and E1B, they cannot replicate in ordinary cell lines or in animal models. The vectors can, however, replicate well in 293 cells, a human cell line that provides the E1A- and E1B-encoded proteins *in trans*.

Although E1A is required for *efficient* induction of transcription of the other adenovirus transcription units, transcription of these genes, including late genes, can occur at low levels in the absence of E1A. Indeed, recent animal model studies and Phase I human trials have indicated that defective adenovirus vectors do elicit an inflammatory response, presumably due to low level expression of adenovirus proteins.

Since we eventually hope to design an adenovirus vector to promote cell death, it will be important to limit the infection *in vivo* to the target tissue, and to minimize infection of healthy tissue. Therefore, the vector should probably be defective. The expression of the 11.6K protein could be limited to the tumor by direct injection into the tumor, or by use of a tumor-specific promoter to drive expression of the 11.6K gene. It is unclear whether the E3 genes should be included to minimize the normal inflammatory response (probably not), or excluded in order to maximize the response; this presumably would depend on the amount of infection of healthy tissue.

The design of an optimal vector is a topic of future experiments. At the present, we will use a simple "first generation" vector to address whether 11.6K expressed essentially alone can kill cells. This experiment differs from that described in Section C.1.a. because the vector will express other adenovirus proteins at low levels.

Our vector will have an Ad5 backbone, and it will be deleted in the E1A, E1B, and E3 regions. The 11.6K gene will be inserted into an expression cassette wherein transcription will be driven by the cytomegalovirus immediate early promoter, and the pre-mRNA will be processed using SV40 polyadenylation and splicing signals. The expression cassette will be inserted into the E1A/E1B region (Fig. 4), and plaques will be picked on 293 cells. Plaques of vector expressing 11.6K should be larger (more cell lysis and virus spread) than plaques from vector lacking 11.6K. Plaques will be expanded into virus stocks, and high-level expression of the 11.6K protein will be confirmed. Cultured human and mouse cells will be infected with the vector, and cell death will be monitored as described in Section C.1.a.

If 11.6K expressed from the adenovirus vector kills cells, then we will proceed with the experiments in Section D. If cells are not killed, then we will perform the experiments in Section C.3.

## **C.2. Mapping the minimal domain of 11.6K that is sufficient to promote cell death.**

Our studies with 11.6K mutants have indicated that a protein consisting only of residues 41-101 (i.e. lacking residues 1-40) localizes quite efficiently to the nuclear membrane and is about 50% as effective as the wild-type 11.6K protein in promoting cell death. A protein lacking residues 79 to 101 is completely wild-type for localization to the nuclear membrane and promotion of cell death. These results suggest that a version of the 11.6K protein consisting

only of residues 41-78 might be sufficient to promote cell death. It is important to determine whether this is so, because such a peptide, synthesized *in vitro*, might by itself be sufficient to promote cell death (e.g. by injecting the peptide into tumors). Also, such a peptide might be less immunogenic than the entire 11.6K protein. We propose to construct an adenovirus mutant that expresses residues 41-78 and determine whether this protein can promote cell death. This mutated gene will be inserted into the *rec700* background, as we have done for all our other 11.6K mutants, and it will be expressed from the *tet*-inducible vector.

### **C.3. What is required in adenovirus-infected cells in order for 11.6K to promote cell death?**

If the experiments described in Section C.1. indicate that 11.6K alone cannot promote cell death, then some other adenovirus gene product or some unknown aspect of the adenovirus productive infection must be required in order for 11.6K to function. The following experiments will be performed to address these issues.

First, we will determine whether 11.6K overexpressed during early stages of infection can promote cell death. The 11.6K gene will be built into our mutant, *dl7001*, which lacks the entire E3 region but expresses all other adenovirus genes. We are using this mutant as a vector to express the E3 proteins individually (Ranheim et al., 1993). Cells will be infected with the *dl7001*-11.6K vector, maintained in cytosine arabinoside in order to inhibit viral DNA synthesis and keep the cells in the early stage of infection, and cell death will be monitored.

Second, we will attempt to map the adenovirus early protein(s) that must collaborate with 11.6K in order for 11.6K to promote cell death. We will infect cells with mutants that lack the E4 region, the E1B region, and the E1A region. We already know that



this putative "collaborating" protein is not encoded by the E3 region. These experiments are similar to those we performed to map genes that function to sensitize cells to tumor necrosis factor (Duerksen-Hughes et al., 1989) and to down-regulate the epidermal growth factor receptor (Carlin et al., 1989). If we determine that an adenovirus late gene encodes the "collaborating" protein then we will attempt to map the gene using available temperature-sensitive mutants.

#### **D. POTENTIAL FUTURE EXPERIMENTS**

The following is a brief description of possible future experiments. Which experiments will be attempted will depend on the results of the experiments in Section C. Most or all of these experiments will probably require additional funding.

##### **D.1. Design of an optimal vector to deliver the 11.6K protein to cells.**

We will attempt to optimize expression of 11.6K in our adenovirus vector, and we will construct vectors with tissue-specific promoters. We will explore whether the E3 region should be included in the vector, and whether a nondefective vector might be useful.

We will also attempt to express 11.6K from a retrovirus vector. Since retrovirus vectors only infect dividing cells, such a vector that expresses 11.6K might be particularly useful to treat brain tumors.

##### **D.2. Delivery of the 11.6K protein by direct injection.**

Since 11.6K has an internal signal-anchor sequence and it has only a short sequence that is C-terminal to the signal-anchor domain, 11.6K may be able to insert into membranes in a posttranslational manner. If so, then liposomes could be an ideal mechanism to deliver the protein to the interior of cells. The 11.6K protein might integrate into the

membrane of the liposomes, become fused to membrane vesicles within cells, and then be targeted to the nuclear membrane via its specific nuclear membrane localization signal. We will attempt this experiment using 11.6K purified from adenovirus-infected cells, where 11.6K is made in large amounts. Alternatively, 11.6K could be obtained from bacterial or baculovirus vectors.

If the experiment described in Section C.2. results in the identification of a minimal domain for 11.6K to function, then the minimal peptide will be synthesized *in vitro*. The peptide will either be incorporated into liposomes or added directly to cells, and cell death will be monitored.

#### **D.3. Coupling of the 11.6K protein to a ligand.**

In one of our adenovirus mutants (*d1718*), the stop codon for 11.6K is deleted; this results in the synthesis of a fusion protein consisting of residues 1-87 in the 11.6K protein fused to residues 14-91 of the E3-10.4K protein. This fusion protein functions nearly as well as wild-type 11.6K in promoting cell death. This suggests that 11.6K could be fused at its C-terminus to some ligand, e.g. EGF, and still remain functional. If so, then the ligand could be used to target 11.6K to specific cell types that express the receptor for the ligand. Thus, the experiment would be to express the 11.6K-ligand fusion protein in bacteria or insect cells, purify the protein, add the protein to the cells, and determine whether the protein is efficiently internalized into cells that express the ligand receptor, and whether the protein promotes cell death.

#### **D.4. Animal models.**

We have found that 11.6K does not localize to the nuclear membrane in

adenovirus-infected mouse or rat cells. This may imply that 11.6K must interact with a human protein in order to promote cell death. Lack of 11.6K function in mouse or rat cells is in one respect unfortunate because an animal model will be necessary to determine whether 11.6K can, for example, cause regression of tumors *in vivo*. We will infect cells of different species, e.g. cotton rat, hamster, and monkey, and determine whether 11.6K localizes to the nuclear membrane and promotes cell death. It may also be possible to examine human tumors growing in immunoincompetent mice. When we find a species where 11.6K is able to promote cell death, then experiments will be done in this animal model.

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*J. Virol.* 52, 307-313.

## FIGURE LEGENDS

Fig. 1. Sequence of the Ad2 E3-11.6K protein. The protein becomes integrated into membranes via its internal signal-anchor domain at residues ca. 41-59. The protein is oriented in the membrane with the NH<sub>2</sub>-terminus in the lumen and its COOH-terminus in the cytoplasm or nucleoplasm (the protein ultimately localizes to the nuclear membrane, but we do not know whether it is the inner or outer nuclear membrane). The site of N-glycosylation is indicated (NTT), as are the major sites of O-glycosylation. A domain rich in basic amino acids and prolines is indicated.

Fig. 2. Schematic representation of the Ad2 11.6K protein, as well as a subset of the mutants constructed in the 11.6K protein. For the wild-type protein (top figure), the major and minor O-glycosylation sites are shown, the N-glycosylation site is shown, the methionines at positions 1, 41, 49, and 56 are shown, the signal-anchor domain is shown, and the basic-proline domain is shown. Also shown is the orientation of the protein in the membrane, with the NH<sub>2</sub>-terminal region in the lumen of membranes, and the COOH-terminal region extending into the cytoplasm and/or the nucleoplasm. The deletions shown for the various mutants are indicated by the schematic. The two right columns in the figure indicate the approximate phenotype of the mutants with respect to their ability to promote cell death, as a percentage of the wild-type protein, and their ability to localize to membranes. Membranes shown in italics are the major sites of localization. NM refers to nuclear membrane, G refers to Golgi. The protein depicted at the bottom of the figure corresponds to the putative proteolytic processing product that arises following initial synthesis of the 11.6K protein.

Fig. 3. A cell viability assay for cells infected with *rec700* (wild-type) or two mutants,

*pm712* and *pm734.1*, which lack a functional 11.6K gene. A549 cells were infected with the viruses, then at different days postinfection cell lysis was measured based upon the release of lactate dehydrogenase (LDH) into the culture medium. The experiment shows that cells infected with *rec700* die (i.e. release LDH) much more rapidly than do cells infected with the two 11.6K mutants.

**Fig. 4.** Schematic of the Ad2 genome. The genome is a linear duplex DNA of 36,000 base pairs. *r* and *l* refer to rightward and leftward transcription, respectively. The split arrows indicate the spliced structures of the mRNAs (exons are shown). 289R, 19K, IIIa, etc. refer to proteins. E1A, E1B, E2 (A and B), E3, E4, and L1 (early) are "early" transcription units that are expressed prior to viral DNA replication. L1-L5 are families of "late" mRNAs expressed in the major late transcription unit. The 11.6K protein is translated from one of the E3 mRNAs at early stages of infection. At late stages of infection, 11.6K is translated from an mRNA that contains the major late tripartite leader (Tollefson et al., 1992).

## **EXHIBIT C**



Kostya, January 30

Project: Develop replicative vectors for cancer gene therapy.

### Progress achieved:

1) 3 rounds of transfection were performed with p626 (pBHG11 with E4 promoter substituted for CMV promoter). No plaques were obtained with this construct or pBHG11 as a control. I have prepared new stock of pAV56 (left-arm plasmid) and repeated the experiment 3 times more. Now waiting for results.

2) The plasmid containing E4 promoter- SPB promoter substitution was constructed (p82). It contains DNA of dl309 from BamHI to NdeI site (E3 region from dl309) and other part to the end of genome from pBHG11 with substitution of E4 promoter. 1 cotransfection experiment was done recently with 1101/1107 cut with EcoRI and pCMV/TTF. *had some TTF plasmid*

3) Cotransfection of 1101/1107 cut with EcoRI and p54 (Lynda's plasmid pdlLKHBam-end with insertion of Ad5 ADP gene) was done. Up to now (26 days) there are no plaques with either 1101/1107/EcoRI or with 1101/1107/EcoRI+ Lynda's plasmid (parental plasmid without insert of ADP). *With p54 there are 7 plaques* The plaques have been picked up and initial stocks of viruses prepared. Now the work is in progress *approx. 2 weeks* to infect monolayers and characterize the genomes of this viruses.

4) I failed 2 times to construct the large plasmid with mutated gp19K on dl309 background. Now *presume* repeating the cloning.

*BDP inserted in XbaI site. Not start in TP.*

### Proposed work:

1) To join Carol's and Mohan's work on 1101/1107 and pm975. I presume it would be interesting to do the same work on primary lung epithelial cells (GTI). SAEC - normal human lung small airway epithelial cells. *GTI get cells from GTI.*

2) Start transfection experiments on Mohan's cell line (293 transformed with pCMV/TTF) to obtain the virus with E4- SPB promoter substitution. Characterize Mohan's 293-TTF cells on their ability to transactivate SP-B promoter (transfection and IP with anti-E4 ORF6 antibodies).

3) Start the work to obtain the plasmid with E1 on 1101/1107 background? Make the point mutation as in pm975? Try to substitute E1 promoter for SPB? Enlarge the CR2 deletion?

*GTI - infect cell lines.*



Jan 31, 97

2

## Outline of Mohan's project:

### 1. C.F. project:

a) plasmid p $\Delta$ E1splA/3.7SPC Rep78 has ~2900 bp deletion. (Gautam's plasmid).

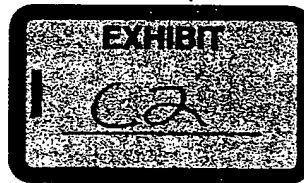
b) Construction of p $\Delta$ E1splA/3.7SPC Rep78 is in progress: Transferring the 3.7 SPC/Rep78 fragment to p $\Delta$ E1splA plasmid. Next week SPC/Rep78.

c) Preparation of high titer BH6 E3TR LacZ virus. <sup>psal assay.</sup>  
 $1.4 \times 10^9$  pfu/ml. Done with 40 dishes.

Looks like virus does not grow well.  
 Try again in KB cell/E1 growing in spinners.

KB/E1 - looks like may grow in spinner.

### 2. Cancer project:



#### a) Experiment 1:

a-1): Growing HEL 299 cells infected with dl1101/1103.  
 Showed CPE ~ 12 days.  
 dl309 showed CPE on 3<sup>rd</sup> day.

a-2): Growth arrested HEL 299 cells infected with dl1101/1107 did not show CPE, till 17<sup>th</sup> day. dl309 showed CPE on 8<sup>th</sup> day.

a-3): IF showed strong presence of fiber and  
DBP in ~ 50% of the growing cells (infected  
with dl1101/1107) on 14<sup>th</sup> and 17<sup>th</sup> day.

b) Experiment 2:

b-1): pm 975 infected growing HEL 299 cells  
showed CPE on 3<sup>rd</sup> day; dl 309 also  
showed CPE on 3<sup>rd</sup> day.

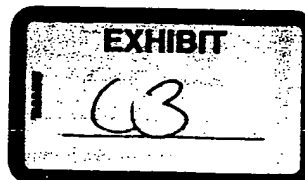
: pm 975 and dl 309 killed all the cells  
~ 6<sup>th</sup> day.

: dl 1101/1107 showed CPE beginning ~ 12<sup>th</sup> day.

b-2): Till 10<sup>th</sup> day → 17 days - still still no CPE  
cells frozen. Next week plaque assay.  
in pm 975 and dl 1101/1107 infected growth  
arrested cells.

dl 309 showed CPE on 8<sup>th</sup> day.

b-3): Growth curve = Growing and growth  
arrested viral infected cells are being  
frozen for plaque assay.



c) Experiment 3:

c-1) HEL 299 growing cells are infected with PM975; dl 1101/1107; dl 309 and mock, at MOI of 100 pfu and 500 pfu/cell. 3<sup>rd</sup> of infection, at 100 pfu PM975 showed moderate CPE and ~90% CPE in 500 pfu infection.

No CPE is seen in dl 1101/1107 infected cells at 100; and 500 pfu/cell. (7<sup>th</sup> day) dl 309 showed CPE on 3<sup>rd</sup> day (100 pfu) and 90% CPE in 500 pfu infection.

Growth arrested cells will be infected today.

Phage assay next week.



Gene Therapy, Group Meeting 5/10/01

Took 1 h 20 min

Karoly 2/17/87

E3 virusesDid not  
make Abp  
ID, 14.7 in  
E1A

- I have made GCL stock of pGluC (D-) 14.7 and NPGK p13K ap
- titrating D under process (NPGK p13K ap  $\approx 10^9$ )
- going to make Ad lac Pol RID and Ad lac Pol 14.7  
RID in E3 region. No base in Cell.
- Bai - CMV-14.7 CMV-RID. Have done. Make min.

FasL projectFasL  
20-transfected 293  
10.4/10.5 plasmid  
pCI (Bai. made)  
also kind.

- I have cloned mouse FasL in pCI, then the CMV-mFL-polyA cassette in a left end plasmid containing p-Gal and VARVAI-II
- Transfected this construct in 293  $\rightarrow$  the cells died (cells don't stay long)
- I could not show mFL in a transient transfection assay (293 cells, Western blot with anti human FasL) but the protein could be detected in an in vitro transcription-translation reaction using the same plasmid and antibody
- Transiently transfected MCF-7 and MCF-7 Fas cells die at the same rate. No cyclo treatment is needed
- I am going to try MCF-7 CmFL first in transient transfections, then make a cell line. This cell line can be used as an "activated CTL" in Fas experiments
- I am making a CmFL-293 line

EXHIBIT

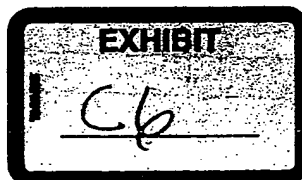
C5

Cancer Therapy project

- It seems that the V138 I use is too high passage. Only a proportion of the cells divide, the majority is senescent. I have ordered a new batch from ATCC.

February 14, 1997 Kostya

- 1) Up to now I have no plaques after transfections with p626 (E4  $\rightarrow$  CMV promoter substitution). And I have no plaques with p8H611 as a control.
- 2) I am preparing now DNA from cells infected with 10 plaques resulting from cotransfection of 1101/1107 DNA cut *EcoRI* with p54 (Lynda's deletion of E3, insert of ADP gene).
- 3) I have plaques after cotransfection of 1101/1107 DNA cut *EcoRI* with p82 (E4  $\rightarrow$  SP-B) promoter substitution. Control (1101/1107 cut with *EcoRI*) to experiment ratio is 3:10 now but in dishes with p82 plaques are still appearing (3 weeks).
- 4) I am working on Moken's cells (293 CMV/TTF), have done one cotransfection of 1101/1107 / *EcoRI* p82 on these cells.
- 5) Cloning experiments to obtain large plasmid suitable for cotransfection with gp191c mutated are in progress.



Feb 14, 97

# Outline of Mohan's Project:

1. C.F. project:
- RNA analysis of Rep78 antisense
  - Hist- photograph of passages (1st x 10<sup>9</sup>) filtered on 293 cells.
- a) Screening colonies for 3.7SPC/Rep78 insert in pXELSP1A.  
36 colonies are under screening; some colonies have the insert; screening more colonies & characterization
- b) SPB promoter = PCR; strategy to clone in front of Rep78.

Mohan - KB/EIA plunger - doing ok. Still in tube (60 ml). Not in spinner yet.  
293 suspension culture. Shows slow. Forms clumps at  $5 \times 10^5$  cells/ml.

## 2. Cancer project:

### Experiment #3:

(growing HEL 299 cells: 14<sup>th</sup> day (2/14/97))

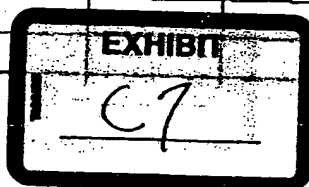
- a) pm 975 (100 & 500 pfu) killed all the cells.
- b) dl 1101/1107 (100 pfu) & CPE starts; in 500 pfu - shows CPE. (better than 100 pfu)
- c) dl 309 (100 & 500 pfu) killed all the cells.

Growth arrested HEL 299 cells: 7<sup>th</sup> day

- a) No CPE in dl 1101/1107 (100 & 500 pfu);
- b) No CPE in pm 975 (100 pfu);  
CPE shows in 500 pfu. Same as 309 at 500 pfu.
- c) No CPE in dl 309 (100 pfu)  
CPE shows in 500 pfu.

Growth Curve:

Dishes are processed; going to do plaque assay



Korohy 2/14/87

E3 viruses

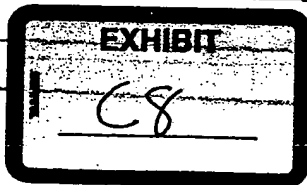
- Titer of N PGK pp13kexp is  $1.37 \times 10^9$
- $\beta$  Gluc(D-) 14.7 is less than  $10^7$  (?) (see repeat titration exp.)
- We are trying to make virus with the plasmids made by Bai
- We are assaying for expression of 14.7 and RID in a Western

FosL project

- I tried the infection-transfection experiment twice. I failed both attempts because the virus killed the cells before I could transfect. I used 50 pfu/cell (233), 20  $\mu$ g/ml AraC. The cells were rounded up after 18h (1st exp.) and 7h (2nd exp.) 5 PFU. MCF7 cells. FosL supernatant. 293/CromA cell line.

Cancer Therapy project

I have got V138 from ATCC, I am growing them up. Got cell proliferation test-stains for Bcl-2.



Kostya

1) I have made and analyzed by restriction and  
PCR ~~to~~ Hirt DNA preps from 5' of 10 plaques  
that I had after p54 <sup>(Linda's plasmid + ADP)</sup> cotransfection with "01/1109/R1".  
2 plaques were wt with addition of something  
that I suggest is defective version of recombinant  
virus. 3 other plaques contain E3 from p54.

2) I'm <sup>Have 10 plaques - but small, but very small. One is with</sup> picking up plaques resulting from <sup>The transfection was complicated.</sup> transfection  
of p82 (E4 → SP-B promoter substitution) <sup>co-transfected G41-  
cut 8/10/1109  
also transfected TFF1.</sup>  
Expt is being repeated - expt is 3 weeks old; Had same plaque.

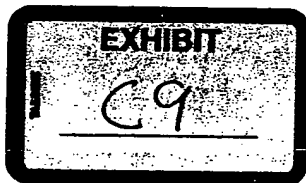
3) Failed to construct large form of gp19K &  
plasmid due to contamination of competent cells  
with something AmpR. Now prepared new batch  
of competent cells, about to repeat cloning.

Ad/ADP recombinant in "Lynnda's plasmid, a E3, + E4, deletion extending like p30041".  
Has 4 plaques, 2 are recombinant (Hirt, PCR), 2 seem to be mix of wt and recombinant  
with a deletion. These plaques give strange appearance, as if they are secreting  
a factor that kills surrounding cells - strain

Lynnda's plasmid - not even sure that G4 is present.

Kostya - will plaque-purify the "mutant" and "mixed" plaques.

→ The two mutant plaques are very "slow" - may not be good tailer virus.





New Emergency Meeting 9:00 AM

(PCI) CMV-internal gene - SV40 polyA  
Koroly 2/21/98

### E3 viruses

- Western with the pCI R1D and CMV-  
was negative - I am doing an IP <sup>in pAETSP1A</sup> 14.7 plasmids  
There are the constructs that Dai made.

### FasL project

- The shedded mFasL is not toxic
- I am trying to make virus with mFasL in left-end plasmid and dl7001

It is known that the <sup>shedded</sup> human FasL is toxic, the <sup>killing by FasL is very fast.</sup> mouse FasL is not.

All MCF-7 cell lines die when transfected with vector/FasL except the one is CrmA.

They die in one day.

25-50% of the cells die.

Will do exp in MCF-7/147K cell line.

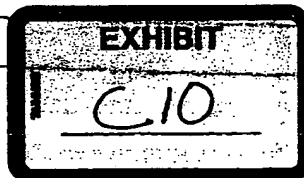
Try any MCF-7 cell line expressing R1D or R1D?

Could try to repeat this exp in A549, even if transient transfection is poor.

Keep making 983 cells expressing CrmA.

MCF-7/CrmA/FasL - have colonies.

- Transfect with R1D + LDNGFP, purity, then transfect with FasL.



## Mohan's project.

Feb 21, 97

## 1. CF. Project:

a) Final plasmid pΔE1Sp1A/3.7Sp1 Rep78 has been constructed; plasmid preparation is under progress.

## 2. Cancer project:

## Experiment #3:

Growing HEL 299 cells 21<sup>st</sup> day

a) d1101/1107 shows better CPE in 500 pfu than 100 pfu.

b) pm 975 & d1309 killed the cells.

Growth arrested: 14<sup>th</sup> day

a) NO CPE in pm975 (100 pfu).

At 500 pfu at 14<sup>th</sup> day cells are killed.  
BPE starts at 3rd day in low light area. Cells start at 12 days.

b) NO CPE in d1101/1107 (100 & 500 pfu)

## Growth Curve:-

pm 975 - infected G & GA. HEL 299 cells.

Growth Curve is done; waiting for plaques.

Will need to do growth curve for d1101 - will do next week.

Northern blot used to done for Rep78 antisense cell line.  
Trying to clone SPB/Rep78 into p.

EXHIBIT

C11

22-28-91 Kostya

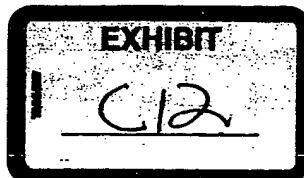
Need to do Hirt on some of these plaques.  
1) I have analyzed by PCR 8 of 10 plaques which I had after 1101/1107 cut with EcoRI + p82 (E4 → SP-B promoter substitution, E3 from dl 309). They all contain wt E4 prom. And I have no up to now plaques with p626 (E4 → CMV promoter substitution, E3 from pBK611). *Determine RHE sequence of Neppan's plasmid.*

1) I am going to put different variants of E3 that I have in ~~small~~ <sup>small</sup> plasmids (dlE3 X60 + ADP, dl 309 with gp19K unmutated, wt?) into Linda's plasmid with which I have obtained a recombinant virus.

1) I would propose to start the work toward E1 promoter deletion and substitution with SP-B or any other tissue-specific or tightly regulated promoters. As alternative we can try to substitute E2A promoter.

1) Growing up ADP mutants vector.

BLAST lipoxigenase - 3 diff species.

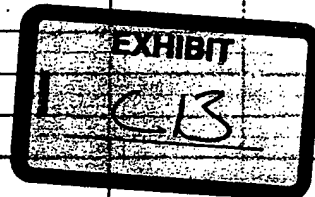


Mohan,

Feb 28, 97,

## 1 C.R. Project:

- a) Preparation of pSEISPIA/SPC Rep78 is progressing. Some problem in CSCI banding. Repairing another prep. Saw 4 bands - good band.
- b) Construction of SPB-Rep78 plasmid.  
: partial digestion of p3.7SPC/3540 with HindIII -



## 2. Cancer Project:

## Experiment #3:

Growing HEL 299 cells 28<sup>th</sup> day1101/1107  
good CPE  
at 500 pfu

- a) d1 1101/1107 shows CPE in 500 pfu and 100 pfu

Growth arrested cells:- 21<sup>st</sup> day.

- a) pm 975 at 100 pfu shows ~ CPE?
- b) NO CPE in d1 1101/1107 (100 & 500 pfu)

Growth Curve:- pm 975 100 pfu. On 293 cells.

Growing:

|                     |   |                   |        |
|---------------------|---|-------------------|--------|
| 3 <sup>rd</sup> day | = | $7.1 \times 10^4$ | pfu/ml |
| 5 <sup>th</sup> day | = | $1.3 \times 10^5$ |        |
| 6 <sup>th</sup> day | = | $1.1 \times 10^5$ |        |
| 7 <sup>th</sup> day | = | $7 \times 10^6$   |        |

Growth arrested:

1101/1107  
any band  
stand  
need to confirm next week.

Need to check 1<sup>st</sup> & 3<sup>rd</sup> day.

|                      |   |                   |        |
|----------------------|---|-------------------|--------|
| 10 <sup>th</sup> day | = | $1.2 \times 10^5$ | pfu/ml |
| 14 <sup>th</sup> day | = | $3.2 \times 10^5$ |        |
| 19 <sup>th</sup> day | = | $1.8 \times 10^5$ |        |

K13/E1A are  
growing. GFP is present  
that proved from 293 cells  
Froze 293 cells  
from microscope.

Keroly 2/28/99

## E3 viruses

- 3 transfection IP with Bai's constructs: I could IP 14.5 (and going. No plasmid yet 10.4?) from an in vitro translated sample. After one day exposure the transfected samples did not show up. The 14.7 plasmids were negative in the in vitro translated samples. Sequencing is under process. 14.7<sup>th</sup>
- 1101/1107 BH611 BH610
- try: BH610
- H610-plasmid on 1101/1107
- made cell stocks of Krigen's two plasmid p-Gal-P6K14.7ap and p-Gal-RDap. I am going to try to make virus with them.

## FasL project

- Have MCF-7-FasL? - I have transduced <sup>partially</sup> R1D and <sup>this morning</sup> mFasL (both in pCI vector) in 293 cells. Result is due this evening tomorrow morning.
- I have infected 293 cells with the CPE stock from the d17001 infection - LENTIL transfection experiment.

## Cancer therapy project

- I use the new 1K138 cells for a new round of infection with 1101/1107.

I need to call Bruce Trapnell. What is real status of money.



3/7/97 Kostya

1) Cloning of ITR PCR product to p82 (EU-SPB promoter plasmid) is in progress.

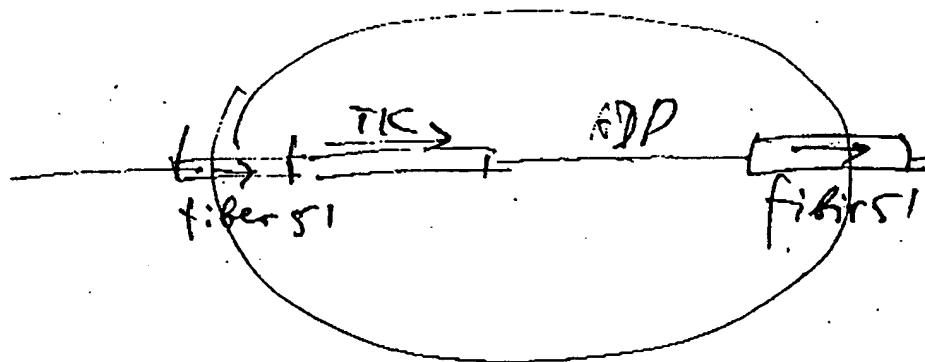
2) I have a problem with TK, cannot PCR it from Linda Morrison's plasmid. If it is possible, I would like to have a plasmid with HSV-1 TK gene from GT1.

3) Plaque assay of 544 (vims with Lyda's E3 deletion + ADP insert) on 293 cells.

309, 1101/1102 (wt control) <sup>Lyda's deletion</sup> 12.5K, 14 polyA, multiple returns

544 develops plaques a bit slower than wt, plaques appeared 1-2 days later than in wt, day 16 - plaques are ~ 8-10 mm in diameter.

7001 - plaques are tiny (1-2 mm) at day 16.



EXHIBIT

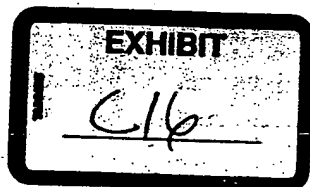
C15

22-141 50 SHEETS  
22-142 100 SHEETS  
22-144 200 SHEETS



Kostya

- 1) i) Results of cotransfection 1101/1107 /EcoRI + p54 (dE3 + ADP) → 4 more plaques were analyzed, 3 of them contain recombinant genomes.  
ii) Subcloning of dE309 E3 with gp19K mutated and dE3 Xba + ADP into Linde's plasmid is in progress. When the plasmid with 19K\* is made I'm going to insert HSV-1 TK into PacI site created in gp19K gene.
- 2) i) No plaques with pG26 (pBAG11 E4 promoter → CMV promoter). No plaques with pBAG11 as a control.  
ii) 4 more plaques resulting 1101/1107 /EcoRI + p82 (E4 → SP-B promoter) were analyzed. All contain wt genomes.  
iii) In 2 repetitions of cotransfection 1101/1107 with p82 (E4 → SP-B promoter) no plaques neither in control nor in experiment, 2nd experiment was done on Moken's 293/CMV-TTF cells. Now repeating the cotransfection.  
iv) Sequencing of Nipper's plasmid p21 (E4 promoter dE) is in progress to prove that right AD ITR is intact in the plasmid.
- 3) Growing initial stocks of the recombinant virus with dE3 + ADP.



Kao 3/7/97

E3 viruses

- I have transfected p-Gal PGK 14.7 exp and p-Gal PGK R10 (with BH611) I am waiting for plaques.
- Still no plaques in Bai's constructs. Now 3 weeks old.

Fest project

- The pC1R10 construct did not protect against killing induced by pC1mFest transfection (293 cells)

Exp 1. - Transfect with 5  $\mu$ g R10

- wait 24h

- Transfect with 5  $\mu$ g mFestExp 2. - Transfect with 5  $\mu$ g R10

- wait 24h

- split the cells

- wait 24h

- transfect with 0.5, 1, 2, 5  $\mu$ g mFest

- The MCF7 14.7 cell line (Terry) is not protected from killing with mFest.

- I am trying to show functional R10 in transfected cells (IP, IF).

- I am ~~also~~ trying to show 14.7 in Terry's line (IF).

- I am expanding colonies isolated from 293cmA and MCF7 cmA-mFest transfections

- I am growing up virus (putative mFest-p-Gal 7001) isolated from blue plaques.

Cancer therapy project

- 11/01/07 on U138 growing 10th day, arrested 7th day - no difference (no CPE). It seems that there might be a diff.

EXHIBIT

C17



Mohan, March 7, 97

## 1. C.F. Project:

APU-ITR - KB/EI growing  
C418A product.

- Preparation of plasmids p $\Delta$ E1sp1A/SPC Rep78 and pB1640: Under dialysis.
- Construction of SPC-Rep78 plasmid - partial digestion + preparative scale.
- RNA blot: For antisense Rep78 RNA in progress.

## 2. Cancer Project:

## Experiment #3:

- Growing HEL 299 cells 35<sup>th</sup> day
- DI 1101/1107 shows CPE in 100 + 500 pfu.

Growth arrested: 26<sup>th</sup> day

- pm 975 at 100 pfu shows CPE.
- No CPE in DI 1101/1107 (100 + 500 pfu)

Growth Curve: DI 1101/1107 (100 pfu) Total cell + supernat

Growing: 5<sup>th</sup> day =  $1.4 \times 10^4$  pfu/ml  
 15<sup>th</sup> day =  $4 \times 10^4$  "  
 24<sup>th</sup> day =  $1.3 \times 10^6$  "

Growth arrested:

5<sup>th</sup> day =  $1.1 \times 10^3$  pfu/ml  
 10<sup>th</sup> day =  $1.4 \times 10^3$  "  
 19<sup>th</sup> day =  $8.4 \times 10^3$  "  
 23<sup>rd</sup> day =  $5.6 \times 10^3$  "

10 309

pm 975 growth  
 Day 1 + 3  
 will be staining  
 next week

Growth Curve: pm 975 Growth arrested days (1 + 3) are titrated.

EXHIBIT

C18

## I E3 region:

i) Initial stocks of the viruses with  $\Delta E3$ , ADP insert are prepared. Plaque assay on 293, A549 cells with 1101/1107,  $\Delta L309$ , ADP<sup>-</sup> viruses as controls is to be started.

ii) Two large plasmids suitable for cotransfection with viral DNA based on Lyda's plasmid with E3 a) from  $\Delta L309$  with  $sp19K$  unmutated b)  $\Delta E3$  Xba + ADP<sup>KO3</sup> are made.

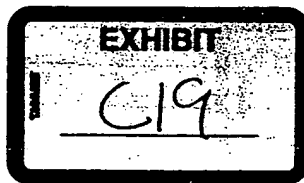
CsCl preps of these plasmids are done. Cotransfection of these plasmids with 1101/1107 DNA / EcoRI is in progress. Will do one for TK gene, and PacI site.

## II E4 - SP-B promoter:

i) There were 4 plaques which are developing CPE very slow in plate wells (Incomplete CPE in 3 weeks).

ii) Alternative project: E1  $\rightarrow$  SP-B (SP-C) promoter substitution. There was an article by David Solnick with E1  $\rightarrow$  MLP substitution (deletion E1 promoter -45  $\rightarrow$  +8 relative to E1 cap site).

However, I would propose to try to express E1A under the control of SP-B or SP-C in place of standard E1 deletion. Advantages: a) E1B would be completely deleted in the virus b) It is possible to use 13S cDNA (express only 289 aa E1A)



Koroly 3/14/

## Fast project

- I did an IF with A543 and 293 cells transfected with pCDNA3 RID $\alpha$ , pCDNA3 RID $\beta$  (alone and together) and pCIRID. The results are inconclusive. I could see <sup>strong</sup> RID $\beta$  expression where it was present, but the localization was mostly Golgi, even if RID was supposed to be there. (This was not easy to judge in 293. The IF did not work in A543). The EGF internalisation did not work because of the antibody. I am going to repeat this experiment in MCF-7.

- I have labeled IP RID $\beta$ , but for co-IP of ROR.
- I have not processed them yet.

- Fast virus: I am doing a plaque assay with the isolated, blue, viruses.

Starting to test 893/crmt clone. Enough antibody? Transfect = Fast - see if

## Cancer Therapy project

- I am doing an IF with 1101/07 infected U138 <sup>DOP, Blue incorporation</sup>
- Growing U138 infected with 500 pfu/cell 1101/07 shows no real cpe on day 15.
- Growing U138 infected with 100 pfu/cell 1101/07 shows cpe on day 3.

1101/1107 does not grow on primary cells. Perfect.

EXHIBIT

C20

Gene Therapy Group Meeting 970314

Mohan, 3/14/97

1. C.F. Project:a) pDEISPIA/3.7 SPC Rept8 has one additional Kho1 site;  
Co-Transfected with pBHG10 in 293 cells.

Give to Fern - labster for E1A and E1B-1A.

b) KB MMT E1 Spinner cells, frozen same, <sup>MTV promoter. Plasmid</sup> all parking =

c) RNA (293 Rept8) - transferred; synthesizing the RNA probe (in progress).

2. Cancer project:Growth Curve : pm 975 Growing:

|                                       |                           |                    |                  |
|---------------------------------------|---------------------------|--------------------|------------------|
| total<br>Cells<br>plus<br>supernatant | Day 1 = $8 \times 10^4$   | pm/ml (New)        | Day 1 x 5        |
|                                       | Day 3 = $7.1 \times 10^7$ |                    | 309 is now being |
|                                       | Day 5 = $1.3 \times 10^7$ | Day 6 - all        | titrated.        |
|                                       | Day 6 = $1.1 \times 10^7$ | all gone floating. |                  |
|                                       | Day 7 = $7 \times 10^6$   |                    |                  |

Growing:

Day 1, d1 1101/1107

Day 3, d1 1101/1107

Day 1, d1 309

Day 5, d1 309

These points  
are being  
titrated by  
stain mixtureGrowth arrested:

GA 1 Day = d1 1101/1107

GA 1 Day = d1 309

GA 5 Day = d1 309

Need to call Bruce.

EXHIBIT

C2

3-21-97

Kostya

1) Sequencing of p21 (Nripen's plasmid, EcoRI-B fragment of pBUG11, EU <sup>12-14 kb</sup> promoter deleted).

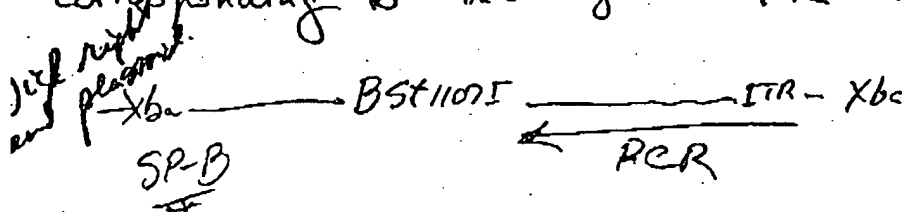
Primers directed to deletion point. <sup>Sall</sup> <sup>SP-C</sup> <sup>SP-B</sup> <sup>CMV</sup>

i) Ad5 sequence is OK ~ 600 bp downstream <sup>the deletion.</sup>

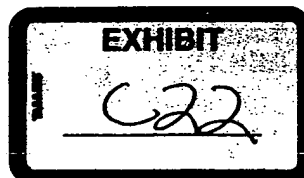
ii) Upstream the deletion Ad5 sequence 35770 → 35858 is as predicted. Upstream of Ad5 35858 nt there is sequence which is <sup>transposon 5.</sup> <sup>in ITR.</sup> *E. coli* Tn5 according to BLAST.

Probably, ITR is in plasmid but it can not be concluded from this sequence. Moreover, even if it is there, Tn5 is inserted - 77 bp from the right end of ITR <sup>Tn5 is at least 500bp or more much longer.</sup> making it not recognizable to virus

2) It is possible to cut out defective sequence from p82 (large plasmid with SP-B promoter) with Bst1107I and XbaI and put PCR product corresponding to Ad5 right ITR in single cloning.



Nripen - site in Gal4 promoter.  
Review Gal4 project.



Karoly 3/25

Fest project

- I have transfected 12 283 crmA clones and got partial protection in 7 of them. I will run a Western blot.
- I have tested 15 MCF7 crmA-mFL clones in a Western blot (using a hFest). All seem to be negative. I will try mixing experiments.
- mFest viruses: no blue plaque in 2nd plaque purification.

Cancer Therapy project

- Growing K138 infected with 100 pfu/cell 11/1/07: no CPE and
- Arrested K138 infected with 100 pfu/cell on 8/5: no CPE and
- Cell proliferation assay (BrdU incorporation  $\rightarrow$  IF) shows that growing K138 is not really growing.

EXHIBIT

C23

Mohan, 3/21/97

1 C.F. Project:

1st transfection was done last week  
 a) 2<sup>nd</sup> transfection was done in 293 cells;  
 pBHG-10 + pAE1Sp1A/3.7 Sp1 Rept8

b) Northern Blot: for 293 / Rept8 cell line, expressing  
 Rept8 Antisense RNA; blot is exposed to film.

c) checking Expression in KB MMTV Et spinner cells. Can.  
 induced by dexamethasone → samples given to Tunder <sup>over</sup>  
 Grow Et/EC cassette, check. Start 5 BTR

2 Cancer Project:

Growing 1 day:  $dl 309 = 4 \times 10^4$  pfu/ml  
 $5^{th}$  day:  $dl 309 = 4 \times 10^6$  pfu/ml

Growth arrested: 1 day  $dl 309 = 1.4 \times 10^5$  pfu/ml  
 $5^{th}$  day  $dl 309 = 1 \times 10^6$  pfu/ml

Growing day 1 =  $dl 1101/07 = 4 \times 10^4$  pfu/ml

Growth arrested day 1 =  $dl 1101/07 = 1.2 \times 10^3$  pfu/ml

Buy spinners from MG

EXHIBIT

C24

## Growth Curve HFL 299 cells

| Growing:        |      | pm 95             | d1 (12)           | d135              | pfu/ml | all (100 pfu)                       |
|-----------------|------|-------------------|-------------------|-------------------|--------|-------------------------------------|
| Day 1           |      | $8 \times 10^4$   | $4 \times 10^4$   | $4 \times 10^4$   |        |                                     |
| " 3             |      | $7.1 \times 10^7$ |                   |                   |        | Repeat @ 500 PFU                    |
| " 5             |      | $1.3 \times 10^7$ | $1.4 \times 10^4$ | $4 \times 10^6$   |        | Do ~ 3-10A - to identify background |
| " 6             |      | $1.1 \times 10^7$ |                   |                   |        | Have CPE data @ 500 PFU             |
| " 7             |      | $7 \times 10^6$   |                   |                   |        |                                     |
| 10              |      |                   |                   |                   |        | Repl - next week @ WI 38            |
| 15              |      |                   | $4 \times 10^4$   |                   |        |                                     |
| 19              |      |                   |                   |                   |        |                                     |
| 23              |      |                   | $1.3 \times 10^6$ |                   |        |                                     |
| 24              |      |                   |                   |                   |        |                                     |
| Growth arrested | Days |                   |                   |                   |        |                                     |
|                 | 1    |                   | $1.2 \times 10^3$ | $1.4 \times 10^5$ |        |                                     |
|                 | 3    |                   |                   |                   |        |                                     |
|                 | 5    |                   | $1.4 \times 10^3$ | $1 \times 10^6$   |        |                                     |
|                 | 6    |                   |                   |                   |        |                                     |
|                 | 7    |                   |                   |                   |        |                                     |
|                 | 10   | $1.2 \times 10^5$ | $1.4 \times 10^3$ |                   |        |                                     |
|                 | 16   | $3.2 \times 10^5$ |                   |                   |        |                                     |
|                 | 15   |                   |                   |                   |        |                                     |
|                 | 19   | $1.8 \times 10^5$ | $8.4 \times 10^3$ |                   |        |                                     |
|                 | 23   |                   | $5.6 \times 10^3$ |                   |        |                                     |

EXHIBIT

C25



Mohan, 1/4/97

1

C.F. Project:

a) 3<sup>rd</sup> Transfection was done; pBHG10 + pAE1sp1a/3.7SPC  
 Next week 18-20 days, should start seeing plaques.  
 (Chemist transfected) On 293

b) Northern blot: 293 cells expressing antisense Rep78;  
 RNA from 293 cells itself, gave hybridization  
 signals (bands) with sense and antisense Rep78  
 RNA probe. May be RNA contamination? or  
 unspecific bands?

293 cells transfected again c Rep78 antisense  
 (pCIS43 Rep78); when the G418 resistant  
 colonies forms, individual colonies will be  
 propagated for the above RNA studies (or PCR).

c) checking expression of EI in KB MMTV EI Spinner Cells.

i) KB MMTV EI cells induced with dexamethasone  
 expressed EI B 19K; for EIA expression the  
 antibody (-Tudor) is not good.

ii) I made a BH6E3 ITR LacZ virus prep from  
 KB MMTV EI Spinner cells (~1.5 liter); so EI is  
 functional in these KB MMTV EI spinner cells.

iii) From 3 liter KB MMTV EI spinner Spinfacer, 8.15 AM  
 dexamethasone for 34 hrs gave viral band same as above

2.

Cancer Project:

Experiment 3: HEL 299 growing and growth arrested  
 cells are infected with dl H2/mt; dl 309  
 (500 pfa/cell) = under progress for  
 Growth Curve experiment

Need graph/table for trip to G.I.



FasL project

- I did a Western with the 293 CmmA clones, they all express CmmA. I will cotransfect them to make FasL virus.
- Use this cell line to make Ad-FasL vector, perhaps Ad-14.7k and Ad-RID vectors.
- Transfect FasL into cells. 1st exp - got protein.

E3 proteins

- I have isolated plaques from CMV RID and CMV 14.7 transfections and V RID and V 14.7 transfections.
- Expression of RIDd from the CMV RID construct is <sup>Neppen RID</sup> <sup>not proven, R→L orientation</sup> not proven yet.

Cancer Therapy

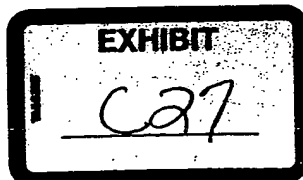
- I am liking up 1101/1107 yields on V138
- There is some CPE in gm875 (100 gm) infected V138 on day 15

Make Ad vector (gene therapy) that expresses CmmA.  
Use for gene therapy to keep transduced cells alive.

Lynne's expression cassette

all promoter, all Ad E3 genes. 1st 293, then MCF-7.  
Do IP

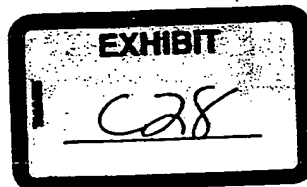
22-141 50 SHEETS  
22-142 100 SHEETS  
22-144 200 SHEETS



4/11/97 Kostya

- 1) Cotransfection of 1101/1107 / EcoRI + p101  
(dl309E3, gp19K mutated) or p111 (dLE3X6a + ADP):  
There are 1 plaque with 101 and 1 plaque with 111  
and no plaques in control dishes. Now amplifying  
these plaques to analyze the genomes.
- 2) Need TK Hsd-I gene from GT1 (if possible, with  
sequence and plasmid map). I'm going to insert  
the TK gene in PaeI site of p101 (in gp19K gene)  
and XbaI site of p111 (~~between~~ upstream from  
ADP insert).
- 3) Failed once to replace Tus sequences with  
ITR in p82 (dLEu promoter, SP-B promoter insert),  
now repeating the cloning.

50 SHEETS  
22-141  
100 SHEETS  
22-142  
200 SHEETS  
22-144



Moham 4/11/97

Gene therapy Meeting1. C.F. Project:

- a) Three different transfections were done to ~~make~~ construct Rep78 virus. No plaques - yet in ~~one~~ transfection (24 days) <sup>transfection.</sup>
- b) Dishes showed CPE like? in ~ 16 days; plaque titrations were done. ~~He started~~ 4-11-97
- c) 293 Rep78, antiseuse cell line were brought from the freezer; going to repeat the above transfections in Rep78 As<sub>2</sub> cell line.
- d) primers are ordered for checking ADV Rep78 in 293 cell line.

2. Cancer project: HEL 299 cells, 500 pfu.

- Growing cells showed good CPE at ~ 15 days (4/10/02)

- Growth arrested cells ~ 11<sup>th</sup> days very little CPE!  
- dishes are being frozen for growth curve data

3. KB MMTVEI:

pBabe puro plasmid is under preparation for ~~co~~-transfection with ~~FI~~ plasmid; pCma plasmids.



Have p21-<sup>gpt</sup>pk, Call ~~Shah~~ <sup>titers</sup>  $1.4 \times 10^7$ 

Keroly 4/11/87

E3 proteins

Do I? of this vector, compare to A15.

Need  
to repeat

- Tested two VPK 14.7 viruses for  $\beta$ -Gal-<sup>one</sup> expresses.
- Tested 3 CMV 14.7 and 4 CMV R10 viruses for  $\beta$ Gal - none of them expresses.
- I have tried to sequence the CMV-R10 construct, did not work. <sup>Lysine-point mutation in active E3 region of A15</sup>  
Need to do this, CMV promoter.

FasL project

- I have a <sup>mixing</sup> ~~binding~~ experiment running with MCF7 - LymA - FasL cells, so far it's a may be.

Cancer Therapy

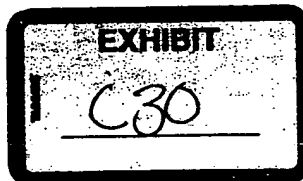
Preliminary titers:

Growing <sup>U138</sup> ~~U138~~ infected with 500 pfu/cell 1101/1107

|                 |           |                   |
|-----------------|-----------|-------------------|
| D <sup>1</sup>  | <         | $10^5$            |
| D <sup>3</sup>  | $\approx$ | $1 \times 10^5$   |
| D <sup>7</sup>  | $\approx$ | $3 \times 10^5$   |
| D <sup>10</sup> | $\approx$ | $2 \times 10^5$   |
| D <sup>15</sup> | <         | $10^5$            |
| D <sup>20</sup> | $\approx$ | $8 \times 10^5$   |
| D <sup>25</sup> | $\approx$ | $1.5 \times 10^6$ |
| D <sup>30</sup> | $\approx$ | $5 \times 10^5$   |

Quiescent U138 infected with 500 pfu/cell 1101/1107

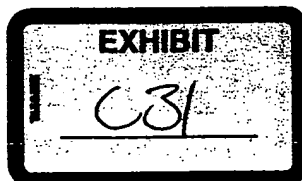
|                 |   |        |
|-----------------|---|--------|
| D <sup>15</sup> | < | $10^5$ |
| D <sup>20</sup> | < | $10^5$ |
| D <sup>30</sup> | < | $10^5$ |

no plaques at  $10^{-5}$  dilution.Will have ~~U138~~ data on U138 next week.22-141 50 SHEETS  
22-142 100 SHEETS  
22-144 200 SHEETS

4/18/97 Kostya

- 1) Plaques, resulting from p101 (gp 19k mutated) and p111 (XbaI(E3 + ADP): I have Hirt preps of genomes of these viruses, now analyzing by PCR and restriction digestion.
- 2) I have got clones with T<sub>u</sub>S → ITR substitution. Going to analyze them.
- 3) Starting to construct plasmids with Ad5, <sup>pm</sup> 760 RID genes under the control of CMV promoter. Same with the 14.7 gene.

Kostya will put E3 transcription unit, containing the pm 794.1 ADP mutations, behind the CMV promoter, for the purpose of inserting into E1 region of BAC-11 (or 10).



1X only 4/10/04

E3 proteins

- I am going to test more MP6K 14.7 and MP6K R10 viruses. (I am infecting today.)
- I did an IP with MP6K p18k virus, no band on ON exposure.
- I have got the oligos for sequencing the CMV R10 construct, I will do it this weekend.

FasL project

- I am growing up 293 cells and making a (SCL) stock of the plasmid (CMV in FasL in left hand plasmid)

Cancer therapy projectPreliminary titers WI-38

|     |          |                   |                   |     |                 |              |                 |
|-----|----------|-------------------|-------------------|-----|-----------------|--------------|-----------------|
|     |          | 1101/1107         | 500 PFU/cell      | 975 |                 | 100 PFU/cell | 308             |
|     | A        | G                 | A                 | G   | A               | G            |                 |
| D1  |          | $1 \times 10^5$   | $1 \times 10^5$   |     | $3 \times 10^6$ |              |                 |
| D3  |          | $5 \times 10^5$   |                   |     |                 |              |                 |
| D5  |          |                   | $1.5 \times 10^5$ |     | $1 \times 10^7$ |              |                 |
| D7  |          | $3 \times 10^5$   |                   |     |                 |              | $3 \times 10^7$ |
| D10 |          | $4 \times 10^5$   | $1 \times 10^6$   |     |                 |              |                 |
| D15 | $< 10^5$ | $< 10^5$          | $2 \times 10^5$   |     |                 |              |                 |
| D20 | $< 10^5$ | $8 \times 10^5$   | $8 \times 10^5$   |     |                 |              |                 |
| D25 |          | $2.5 \times 10^6$ | $7 \times 10^5$   |     |                 |              |                 |
| D30 | $< 10^5$ | $7 \times 10^5$   |                   |     |                 |              |                 |

EXHIBIT

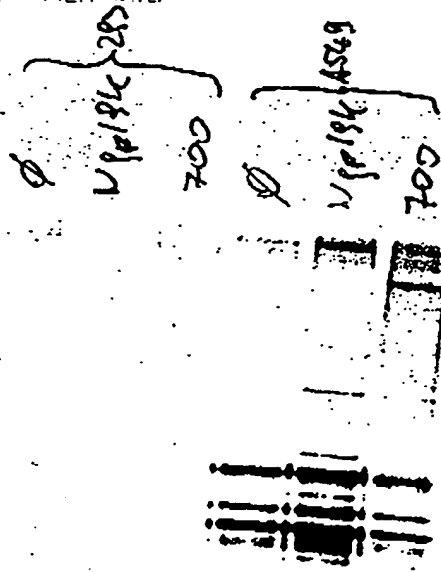
C32

 22-141 50 SHEETS  
 22-142 100 SHEETS  
 22-144 200 SHEETS


KODAK SAFETY FILM

KODAK SAFETY FILM

4/18



Could see by IF, beginning at 2 drop pi

EXHIBIT

C33



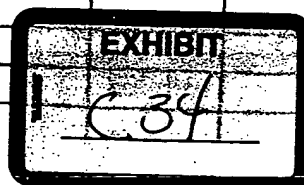
Moham, 4/18/97

1. C.F. Project:

- a) No plaques in 293 cells co-transfected with pBHG10 and pAE1Sp1A/3.7 SPC Rep78. (~ 30 days and 24 days); 3<sup>rd</sup> transfections (18<sup>th</sup> days) dishes are stained; waiting for plaque plaques.
- b) Cells showed CPE like effect (pBHG10 + pAE1/SPC Rep78 were titrated and not stained yet. But even undiluted dish did not show CPE.
- c) RT-PCR showed no Rep78 dist fragment in 293 and 293 Rep78 AS cell lines.
- d) Spinner KB MMTV ET cells - Virus IR LacZ is ~~under~~ in spinner; Karl virus is going next.
- e) pBabe puro plasmid is made in large quantities; for transfecting the KB ET cell lines with ET and CMV A plasmid separately.

2. Cancer project - HEL 299 cells; 500 pfu  
 - Growing cells were dead at ~ 21 days.  
 - Growth arrested cells 18<sup>th</sup> day. On 21<sup>st</sup> day cells will be frozen for Growth Curve analysis.

3. preparations: plasmid BHG10, BHGE3, pAE1/SPC Rep78; will be CsCl purified.  
 293 Rep78 AS cell line will be co-transfected with  
 BHG10  
 BHGE3 + pAE1 Sp1A/3.7 SPC Rep78 plasm  
 AEG DNA  
 plasmids





4/25/97 Kostya

23-141 50 SHEETS  
23-142 100 SHEETS  
23-144 200 SHEETS

- 1) I have got the virus 1101/1107 with gp19k mutated (restriction digestions and PCR).
- 2) Now preparing Hirt's from 4 plaques which should be 1101/1107 E1; Xba E3 deletion, ADP insert.
- 3) Failed to substitute Tus → ITR. Going to do it in 2 clonings but without partial digestions.
- 4) Sequencing shown that ITR is OK in Lyda's plasmid; could not sequence through ITR-ITR junction in pB610, 11; I have repeated the sequencing with different PCR protocol, now waiting for results.
- 5) I have checked with Ann, she doesn't have plasmids with rec 700 or pm 734.1 which would be suitable to cut out SrfI - NdeI fragment corresponding to whole E3, but she does have the viruses. Now going to prepare rec 700 and pm 734.1 DNA to insert SrfI - NdeI fragment to pCDNA3 / EcoRV site.
- 6) The virus 544 (1101/1107 E1, Lyda's deletion of E3 ~~1101/1107 E1~~, 12.5 K ATG mutated, d127984-28153 d128390-30883) + ADP insert into PaeI site in place of second deletion. → I gave the virus to Shari to grow up Cse prep.

Need TK gene from GTI.



Keroh 4/25/97

E3 proteins

- I have tested more NPGK 147 and R1D viruses. The ones that stain for p-Gal show some staining in IF for 14.7 or 14.5. The R1D  $\beta$  staining shows the same pattern as the previously made ~~Ad~~ Ad R1D viruses. In addition to this, the cells that stain are dead ~~for~~ (carryover from 293T). The 14.7 staining looks normal. I am growing up more of the positive clones.

- FesL project

- I have two transfections going in 293 Crn A.

Cancer Therapy project

- I have infected MCF-7 with 01/07, 975 and 308. Will do the same with A549 and 293 and take a growth curve.
- I am infecting W138 for the missing timepoints.
- I am titrating the remaining samples.

50 SHEETS  
100 SHEETS  
200 SHEETS

EXHIBIT

C37

1101/1107

175

308

A

G

A

G

A

G

D1

D3

D5

D7

D10

D15

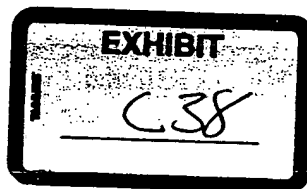
D20

D25

D30

 $< 10^5$  $2 \times 10^5$  $< 10^5$  $1 \times 10^5$  $5 \times 10^5$  $3 \times 10^5$  $4 \times 10^5$  $< 10^5$  $8 \times 10^5$  $2.5 \times 10^6$  $7 \times 10^5$  $1 \times 10^5$  $1.5 \times 10^5$  $1 \times 10^6$  $2 \times 10^5$  $8 \times 10^5$  $7 \times 10^5$  $4 \times 10^5$  $3 \times 10^5$  $1.4 \times 10^7$  $1 \times 10^6$  $1 \times 10^6$  $3 \times 10^6$  $1 \times 10^7$  $6 \times 10^7$  $2 \times 10^7$  $< 10^5$  $2 \times 10^7$  $4 \times 10^7$ 

22-141 50 SHEETS  
22-142 100 SHEETS  
22-144 200 SHEETS



Sheet1 Chart 2

Approximate titers in WI38 4/25/97

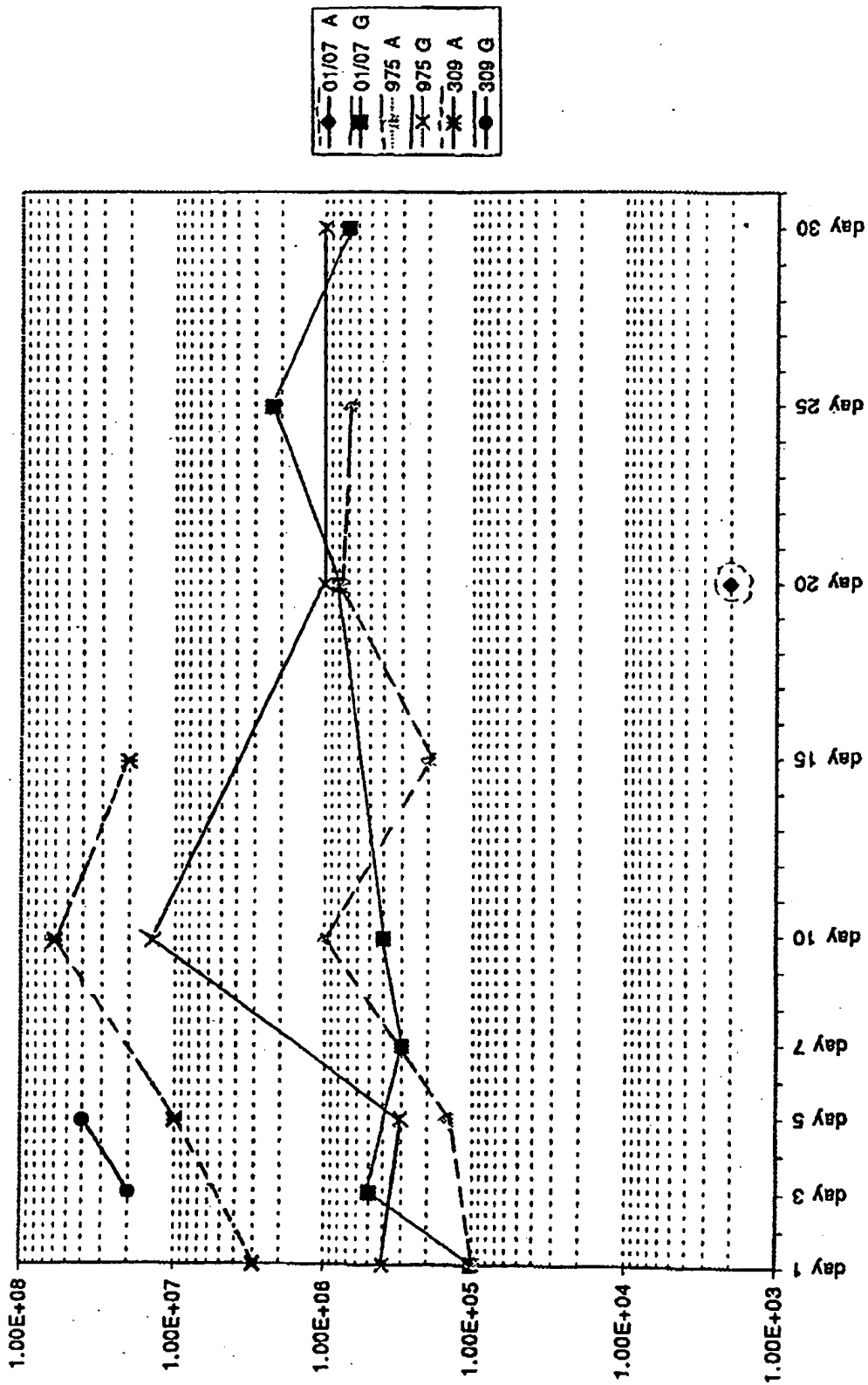


EXHIBIT  
C39

Nohan, t/25/97

1. C.F. Project:

- a) cells showed CPE like effect (co-transfected pAE1SP1A/3.7SPC Rep78 and pBHG10) were titrated. No plaques yet 15<sup>th</sup> day. But when 293 cells infected with CPE, CPE like started look Ad CPE typical.
- b) In 293 Rep78 AS - cell line, co-transfections of pAE1SP1A/3.7SPC Rep78 and pBHG10 were done; pAE1SP1A/3.7SPC Rep78 and pBHG10E3 plasmids.
- c) Virus Ct 14.7 are growing in KB MNTU E1 Sp144 cells.
- d) GAPDH (Glyceraldehyde 3Phosphate dehydrogenase) primers - ordered.
- e) pBHG10, pBHG10E3 under preparation for further transfections.
- f) I am going to isolate d1700 DNA for transfection → Virus Rep78.

2. Cancer Project: HEL 299 cells

- Growing and Growth arrested cells are from for growth Curve analysis.

EXHIBIT

C40

22-141 50 SHEETS  
22-142 100 SHEETS  
22-144 200 SHEETS

9/2/97 Kostya

- 1) Terry's gp19K plasmids. pCR11gp19K Ad5 turned out to be pCR11gp19K Ad2. pC13gp19K contains gp19K Ad2 in correct orientation. (Karol - Transfection - 1P).
- 2) Cloning of the complete E3 under CMV promoter:
  - i) There was E3 inton in Horwitz's virus, their construct starts ~200 bp after E3 transcription start in Ad2 E3.
  - ii) Started to extract Rc 700 and pm 734.1 (m1m41) genomes to clone E3 to pCDNAB (or pC1?).
- 3) Cloning experiments to replace ITR in SP-B ↔ E4 promoter plasmid are in progress.
- 4) Got the virus 1101/1107 E1, <sup>grows much better than d17001</sup> d1E3 X6a + ADP. Checked with restriction digestion and PCR. Recently started experiment (plaque assay) to compare growth properties of 3 recombinants (Lynda's deletion E3 + <sup>KD1</sup>ADP; d1309 gp19K <sup>KD2</sup>untated; d1E3 X6a + <sup>KD3</sup>ADP). Using d1309; 1101/1107; pm 734.1 (m1m41); 7001 as controls. Cell lines - 295 and A549. Going to start with 1P to check for ADP expression. I'm really ready to put TK into 2 last recombinants E3, need TK plasmid and TK sequence from GT1.  
Need TK from GT1!





Kashy 5/2/87

ES proteins

- I am growing up NPGK 14.7 #5 stained best IF.
- I did an IP with transfected p13k and 14.7 constructs (in 283 cells). All but pCBK3 14.7 expressed, all comparable to virus injection. (Labeling 38h post IF and 18h p.i. for 3h)
- I did an IF with the same transfections (in MCF7 cells), it did not work, I am repeating it.

lacZ pGK-14.7  
Moln got and will  
bead.

FasL project

- I am waiting for fagus in 293-Crns cells.  
Have one plaque after 10 days.

Cancer Therapy project

|                 |      |                      |                    |                    |
|-----------------|------|----------------------|--------------------|--------------------|
| I have infected | MCF7 | 01/07<br>500 $\mu$ m | 975<br>100 $\mu$ m | 309<br>100 $\mu$ m |
|                 | A548 | 500 $\mu$ m          | 500 $\mu$ m        | 500 $\mu$ m        |
|                 | 283  | 500 $\mu$ m          | 100 $\mu$ m        | 100 $\mu$ m        |

283 is rounded up 1 day p.i.  
A548 2 days p.i.  
MCF7 5 days p.i.

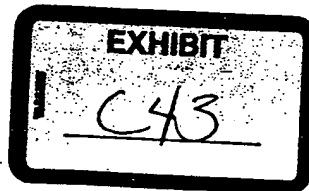
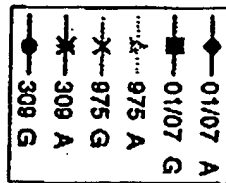
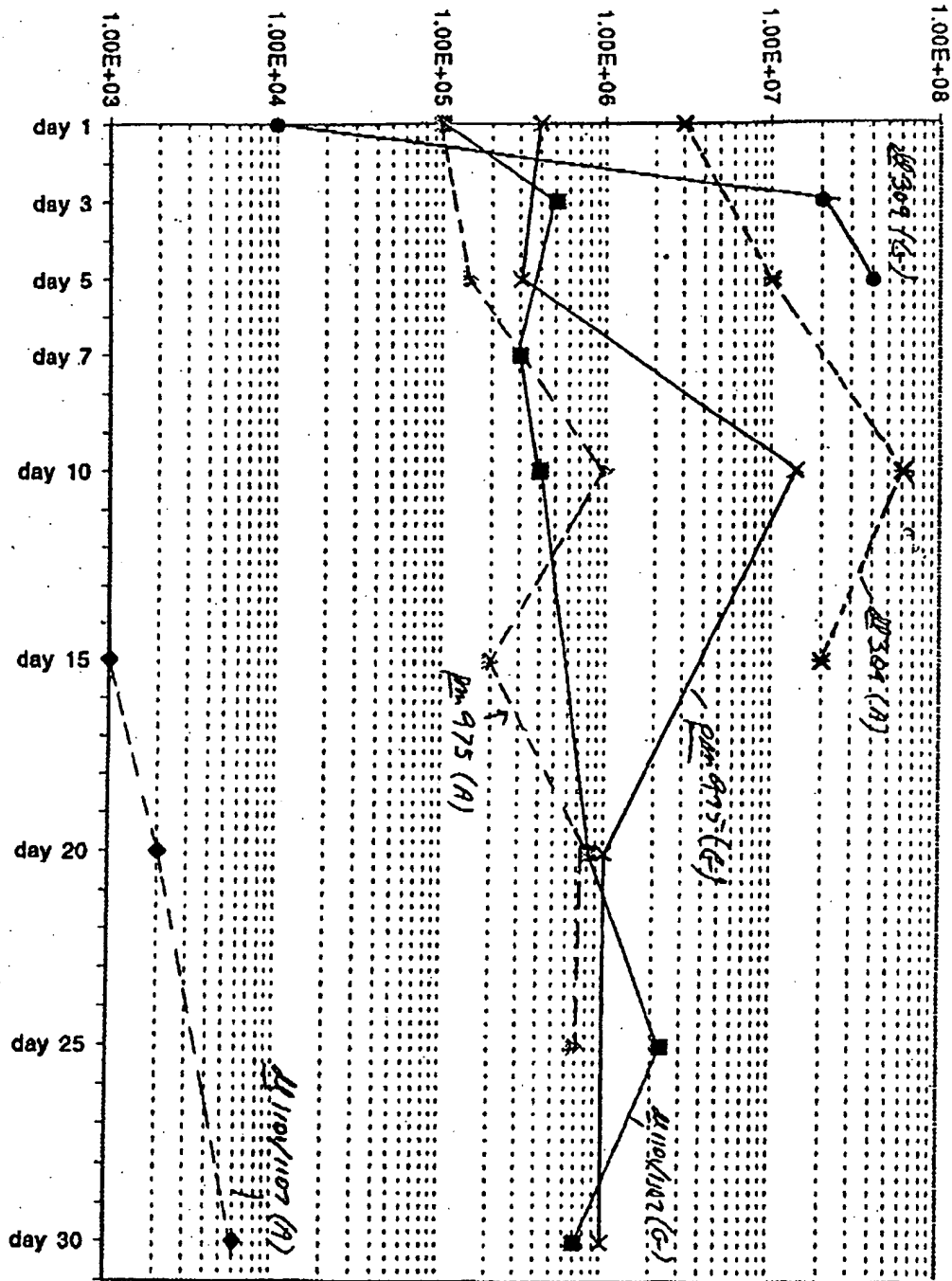
22-141 50 SHEETS  
22-142 100 SHEETS  
22-144 200 SHEETS



EXHIBIT

C42

Approximate titers in W138



5/2/97 Kostya

- 1) Terry's gp19K plasmids. pCR11gp19K Ad5 turned out to be pCR11gp19K Ad2. pC1gp19K contains gp19K Ad2 in correct orientation. (Karol - Transfection - IP).
- 2) Cloning of the complete E3 under CMV promoter:
  - i) There was E3 inton in Horwitz's virus, their construct starts ~200 bp after E3 transcription start in Ad2 E3.
  - ii) Started to extract 700 and pm 734.1 (m1m41, genomes to clone E3 to pCDNAB (or pC1?).
- 3) Cloning experiments to replace ITR in SP-B ↔ E4 promoter plasmid are in progress.
- 4) Got the virus 1101/1107 E1, <sup>grows much better than dl7001</sup> dlE3 X6a + ADP - Checked with restriction digestion and PCR. Recently started experiment (plaque assay) to compare growth properties of 3 recombinants (Lynda's deletion E3 + ADP; dl309 gp19K mutated; dlE3 X6a + ADP). Using dl309; 1101/1107; pm 734.1 (m1m41); 7001 as controls. Cell lines - 293 and A549. Going to start with IP to check for ADP expression. I'm really ready to put TK into 2 best recombinants E3, need TK plasmid and TK sequence from GT1. Need TR from GT1!

22-141 50 SHEETS  
22-142 100 SHEETS  
22-144 200 SHEETS



1. C.F. Project:

- a) BHCE3172 LacZ virus gave moderate band in CSCI banding. But the  $\beta$  gal activity/ml is  $\sim 1.4 \times 10^9$  which is same as previous 172 LacZ preparation. Second set is being titrated to compare the pfu/Lfu/ml.

[ I may use Centricon 100 (M.Wt cut off 100,000) to concentrate the viral stocks for one CSCI spinning which will increase the pfu. Dialysis of stock 172 LacZ virus will increase the volume and it is hard to spin in one tube.

- b) Co-transfection of pE-Sp1A/37 Src Rep78 & pB2610; pB26E3 in 293 Rep78 antisense cell line will be stained next week.

2. E3 project:-

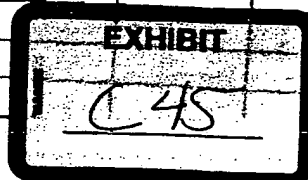
- a) Virus C+14.7 (pCK $\pi$  14.7 + p gluc) gave moderate band in CSCI spinning. Viral titrations are in progress.

- b) Going to make another virus 14.7 LacZ (pCK 14.7 in E3; LacZ in E1) preparation. ~ infection to day

3. Cancer Project:-

Growing and growth arrested virus infected HEL 293 cells ~~extract~~ extract will be titrated for growth curve next week.

1101/1107 at 500 PFU/cell  
309 11 " "



Sheet1 Chart 3

Growth curve 3

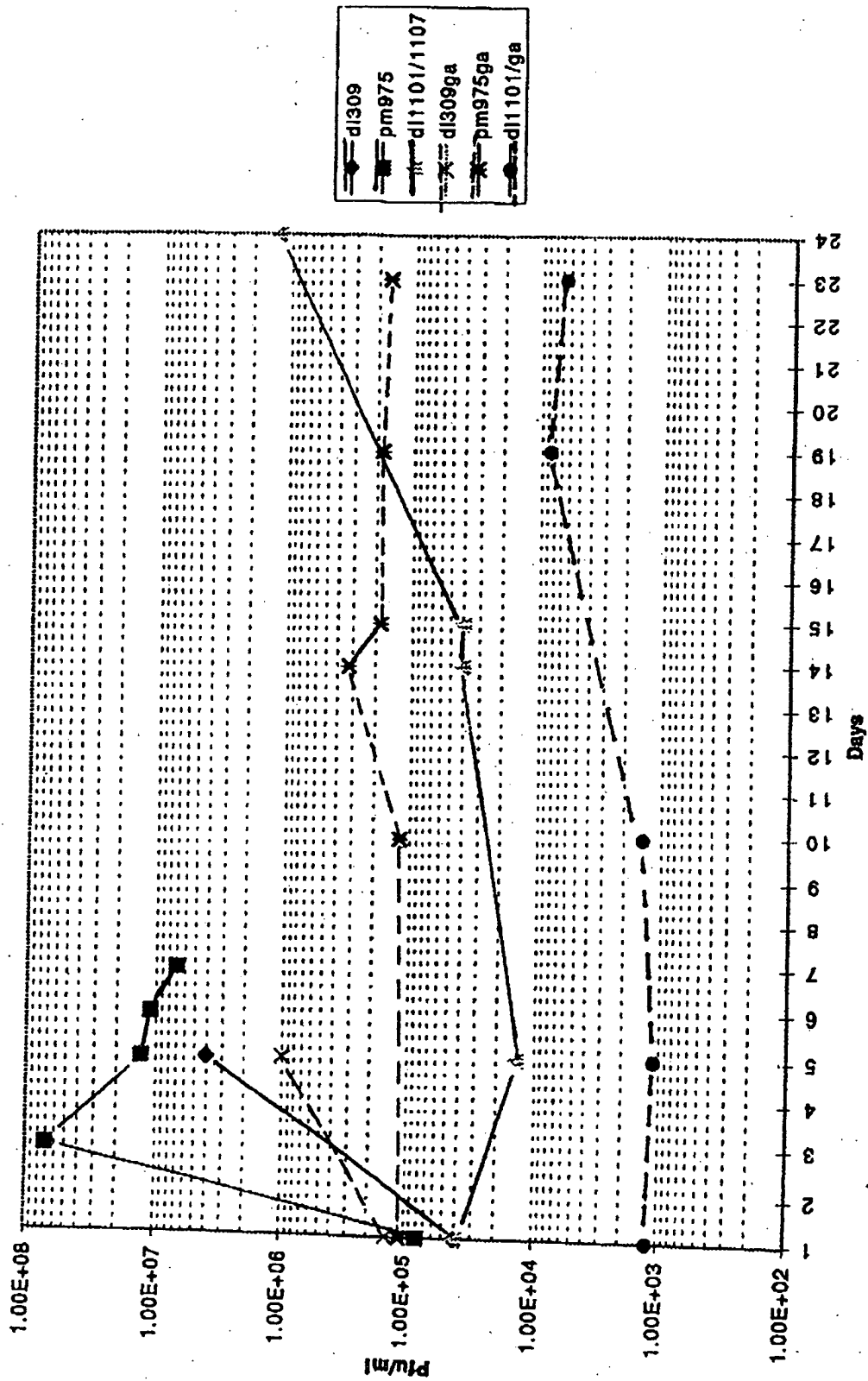


EXHIBIT  
C46

Kostya

1) Results of cotransfection 1101/1107 / EcoRI + p54 (dlE3 + ADP) →  
4 more plaques were analyzed. 3 of them contain recombinant  
genomes.

ii) Subcloning of dl 309 E3 with gp19K mutated and  
dlE3 Xba + ADP into Linde's plasmid is in progress.  
When the plasmid with 19K\* is made I'm going to  
insert HSV-1 TK into PacI site created in gp19K gene.

i) No plaques with p82B (pBAG11 E4 promoter → CMV promoter).  
No plaques with pBAG11 as a control.

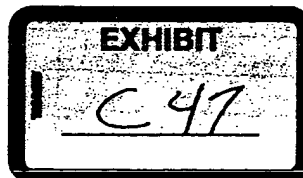
ii) 4 more plaques resulting 1101/1107 / EcoRI + p82 (E4 → SP-B  
promoter) were analyzed. All contain wt genomes.

iii) In 2 repetitions of cotransfection 1101/1107 with  
p82 (E4 → SP-B promoter) no plaques neither in control no in  
experiment. 2nd experiment was done on Moken's  
293/CMV-TTF cells.

Now repeating the cotransfection.

iv) Sequencing of Nipper's plasmid p21 (E4 promoter dl)  
is in progress to prove that right Ad ITR is intact in  
the plasmid.

i) Growing initial stocks of the recombinant virus with  
dlE3 + ADP.



Gene Therapy Group

Koroly 5/8/97

E3 proteins

- I am growing up MP6k 14.7 #5
- I did an IF with transfecto 13k and 14.7 constructs in MCF7 cells, all of them but pCDNA3 14.7 expressed well.
- MP6k gp13k and pGlc 14.7 were negative after 20h.
- Nelson is doing a longer infection assay

Forsk project

- I have 4 plaques. They appeared at 10-11 days after transfection with BTHG11 in 293 crm A cells. Will pick one plaque (large) today.

Cancer Therapy project.

293, MCF7, lost time mistake = must pick Repeat = 293 A549...

EXHIBIT

C48

I am repeating A548 infections and some U138 infections.

CPE in U138 cells

A = 0.2% serum FCS  
G = 5% FCS

01/07 A

no CPE

01/07 G

atypical CPE starting around day 10

No CPE = 1101/1107, either in growing or non-growing.

500 pfu

The "growing" don't really grow.

But, have fewer cells in the growing than non-growing

2 photographs as done with 0 PPM.

last week 12 plaques A - cat to test for free EBF 38, 112-219 mixing ratio 11 cells 1000.

975 G  
D2 +/-  
D3 +/-  
D4 +  
D5 ++  
D6 +++

No real diff. between G and A for 975 500 PFU

975 A  
D4 +/-  
D5 +  
D6 ++  
D7 +++

500 pfu  
For 100 PFU, growing was same as 500 PFU. But, arrested did not show CPE until ~15 days. Same = HEL-299.

308 G  
D3 +/-  
D4 +  
D5 ++  
D7 ++

308 A  
D5 +  
D6 +  
D7 +

100 pfu

also pCI  
- Bay pCDNA3.1. Clone 9P19, 14.7, RLD  
- M12-14.7 - M12-14.7A.

50 SHEETS  
100 SHEETS  
200 SHEETS22-141  
22-142  
22-1445/9/97 Kostya

- 1) Construction of the plasmids with whole E3 (rec 700, pm 734.1) is in progress.
- 2) I have constructed the plasmid with E4 $\leftrightarrow$ SP-5 where Tn5 $\leftrightarrow$  ITR, sequenced the plasmid, now waiting for sequencing results to make the large version of the plasmid for cotransfection.
- 3) For 101-1 (dl 3.4 E3, gp 19K untested) plaques are appearing the same day as for 309. (day 4)  
For 111-1 (E3 XbaI dl + ADP) plaques appeared 1 day before dl 309. (day 3)

① KD-1. 1101/1107 in Lynda's E3 background + ADP  
No promoter for ADP.

Shari harvested day 2 pi, got band, well titer.  
Cells were beginning to die. Shari will titer.

② KD-2 1101/1107, 309 E3, gp 19K untested.  
Plaques appearing same day as 309.

③ KD-3 1101/1107, XbaI deletion (dl 327 deletion),  
ADP without promoter, but in-frame c. 6.7K.  
Got plaques 1 day before dl 309 (i.e. on day 3).  
Got this <sup>one</sup> plaque 4 days posttransfection, 5mm wide.  
Got other 3 plaques 7 days posttransfect  
DNA screened - are recombinant.

Future - could put ADP under control of SPB promoter.  
First, concentrate on what we have.

inject pADP-ADP into 29.3 cells





Mohan, 5/9/97

1. C.F. Project:

- a) Virus BM6 E3 ITR LacZ gene  $1.4 \times 10^9$  Lfu/ml (preparatory)  
 Plaque assay gave  $9 \times 10^8$  pfu/ml (10th day)

I received Centricon 50 concentrator from Millipore as  
 samples - going to concentrate the ITR LacZ virus.

- b) So far no plaques for constructing Rep78 virus.

- c) GAPDH - RT-PCR in progress.

- d) Going to transform d1700 pMX (lat digested, with  
 pSP6 Rep78 plasmid).

2. E3 project:

Dactin out convention, left-to-right orientation

- a) Virus C+14.7 (p~~14.7~~ <sup>pol II</sup> 14.7 + p glue) has been  
 Titrated. No plaques yet - 10th day.

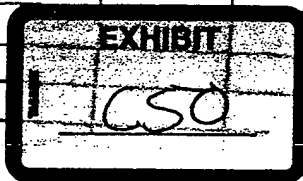
- b) CPE stock for 14.7 LacZ (p~~14.7~~ <sup>pol II</sup> 14.7 + LacZ) virus  
 has been made. Going to titrate (5ml final)

- c) A549 cells are infected  $\pm$  700, GP19K and  
 C+14.7 virus for i.f. (2 days & 4 days)  
 Hard 2 days - negative already infected.

3. Cancer project:- HEL 293 cells

Growing, growth arrested, d1. 11/01/07 (500 pfu)  
 infected - CPE stocks are Titrated for growth curve  
 Will do 309 in coming week

HEL-293 100, 500 PFU Graph: days p.i. vs CPE - will give me.



5/23/97 Kostya

1) E3 project:

Cloning of E3 from rec 700 and pm 734.1 in pBSSK (+) - got bacterial colonies, analyzing then reclone into pCET, then make virus.

2) Cancer therapy project:

- i) Made a large plasmid (Bam → end of genome) with E4 → SPB promoter and ITR repaired, now making CaCl prep. of the plasmid to transfect.

Next week make virus

- ii) Plaque development assay for E3 ADP viruses:

## a) 293 cells (day 18)

Negative control:

7001 - plaques just started to appear (day 18).

734.1 - plaques appeared day 14-15

Positive control:

309 - day 4-5

1101/1107 - day 4-5

Recombinant viruses:

KD1 (544, LKH d1 E3 + ADP) - day 5-6, plaques smaller than wt.

KD2 (101-1, d1309, gp 19K \*) - day 4-5, large plaques.

KD3 (111-1, d1327 + ADP) - day 4-5, large plaques.

## b) A549 cells (day 8)

Same day a little faster than wt. Perhaps a little larger.

309 - day 4-5 (large)

1101/1107 - day 8 (small)

734.1; 7001 - no plaques yet.

KD1 - didn't make

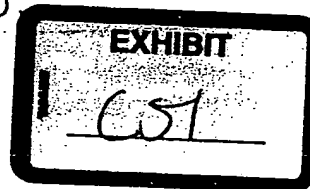
KD2 - day 5-6, large, smaller than wt. (309)

KD3 - day 5-6, smaller than KD2.

positive  
control

negative  
control

recs.



Mohan, 5/23/97

1. C.F. Project:

- a) Virus BH6531TR LACZ was concentrated using Centricon filter columns. Lfu is  $\sim 3.86 \times 10^{10}$ /ml. (1 ml)
- b) d1700 viral DNA digested with ClaI and pAE1Sp1A/Sp1Rep were co-transfected.
- c) 293 TTF cell lines were transfected with pAE1/Sp1Rep. I.F. stained for Rep78. Most of cells transfected with pAE1/Sp1Rep78 are washed out. Few cells (about 2) showed nuclear staining. But not clear.
- d) 293 L Rep78 antisense cell lines showed GAPDH-PCR fragment not the Rep78 fragment. So, no LAV2 contamination.

2. E3 Project:

- a) NPGK LACZ 14.7 <sup>(CPE strain)</sup>  $\text{gand} = 3 \times 10^8$  Lfu/ml in 293 cells  
but in A549  $= 2.3 \times 10^8$  Lfu/ml.  $\sim 10$  fold more in A549 cells than 293 cells.

- b) I.F. = A549 cells: 1700  $\sim 21$  hrs (21 hrs) for 14.7  
 $\sim 50$  pfu/cell NPGK 14.7 - 1 day -  
2 day -  
4 day + + + +  
(Bactin P1) 14.7 - 4 day + + (cytoplasmic staining)  
GP19K = Background +

Virus 14.7 NPGK 14.7 expresses 14.7 at 4 days post infection at wt. level.

Ct 14.7, at 4th day expresses lesser. Mainly cytoplasmic staining and around the nucleus.

Ct 14.7 when titrated  $10^7, 10^6, 10^5$  no plaques. - Titer may be less. So, -IF- may be less.

EXHIBIT

CS2

3 Cancer project: HZL 299 cells 500 pfu/cell

Growing

div 309 div 100/07

|       |                   |                   |
|-------|-------------------|-------------------|
| 3 hrs | $9 \times 10^3$   | $2 \times 10^4$   |
| 1 day | $1 \times 10^4$   | $4.2 \times 10^4$ |
| 4 "   | $1.6 \times 10^4$ | $1.2 \times 10^5$ |
| 6 "   | $2.3 \times 10^4$ |                   |
| 8 "   | $9 \times 10^4$   | $1.4 \times 10^5$ |
| 10 "  |                   | $7.1 \times 10^5$ |
| 15 "  |                   | $2.2 \times 10^6$ |
| 21 "  |                   | $4.6 \times 10^6$ |

Growth arrested

|       |                   |
|-------|-------------------|
| 3 hrs | $4 \times 10^4$   |
| 1 day | $4.4 \times 10^4$ |
| 4 "   | $4 \times 10^4$   |
| 8 "   | $5 \times 10^4$   |
| 10 "  | $1 \times 10^4$   |
| 15 "  | $1 \times 10^4$   |
| 21 "  | $2 \times 10^4$   |

EXHIBIT

CSS

Landy 5/23/97

ES proteins

- I am doing an IP with NPGK K<sub>7</sub> and Ct K<sub>7</sub>
- I am growing up lac-Pol II p19K
- I am cloning the MTZ expression cassette in pCI2lac (hyuda changed the BamHI and BspII sites to PacI in pCI)

Fos L project

- I have isolated 4 plaques. <sup>Could be WT.</sup> They grow very fast (kill 293 ON, show CPE in A549 ON).  
I have infected MCF7 and MCF7crmA cells, it shows CPE ON in both.
- I have tried to PCR out Fos L from the virus, it failed. Positive control (plasmid) was OK.
- The virus expresses  $\beta$ -gal
- I am trying to show the protein in IF.

Cancer therapy project

- I am going to test yields from A549, MCF-7 and 293 next week.

22-141 50 SHEETS  
22-142 100 SHEETS  
22-144 200 SHEETS

5/30/97 Kostya

1) E3 project:

Made the plasmid with E3 from pm 739.1 (u1/u41).  
(ADD<sup>-</sup>) - Sfi1-Nhe1 - fragment in pBlueScript. Now cloning  
is in progress to put it into pCDNA3.1zeo(+)- new  
plasmid with CMV promoter. Now just started.

2) Cancer therapy project:

i) Made cotransfection of "01/11.07" (EcoRI with <sup>Bam</sup> p152SPB<sup>in</sup>  
(new version of large plasmid Bam - ~~end~~ with <sup>Et</sup>  
S0B  $\leftrightarrow$  E4 promoter substitution). to 293 and promote  
293-TTC cells, cotransfected pCMV/TTC as well. Has ITR  
(made by Mohan)

ii) Plaque development assay for E3 ADP viruses:

293 cells - see graph.

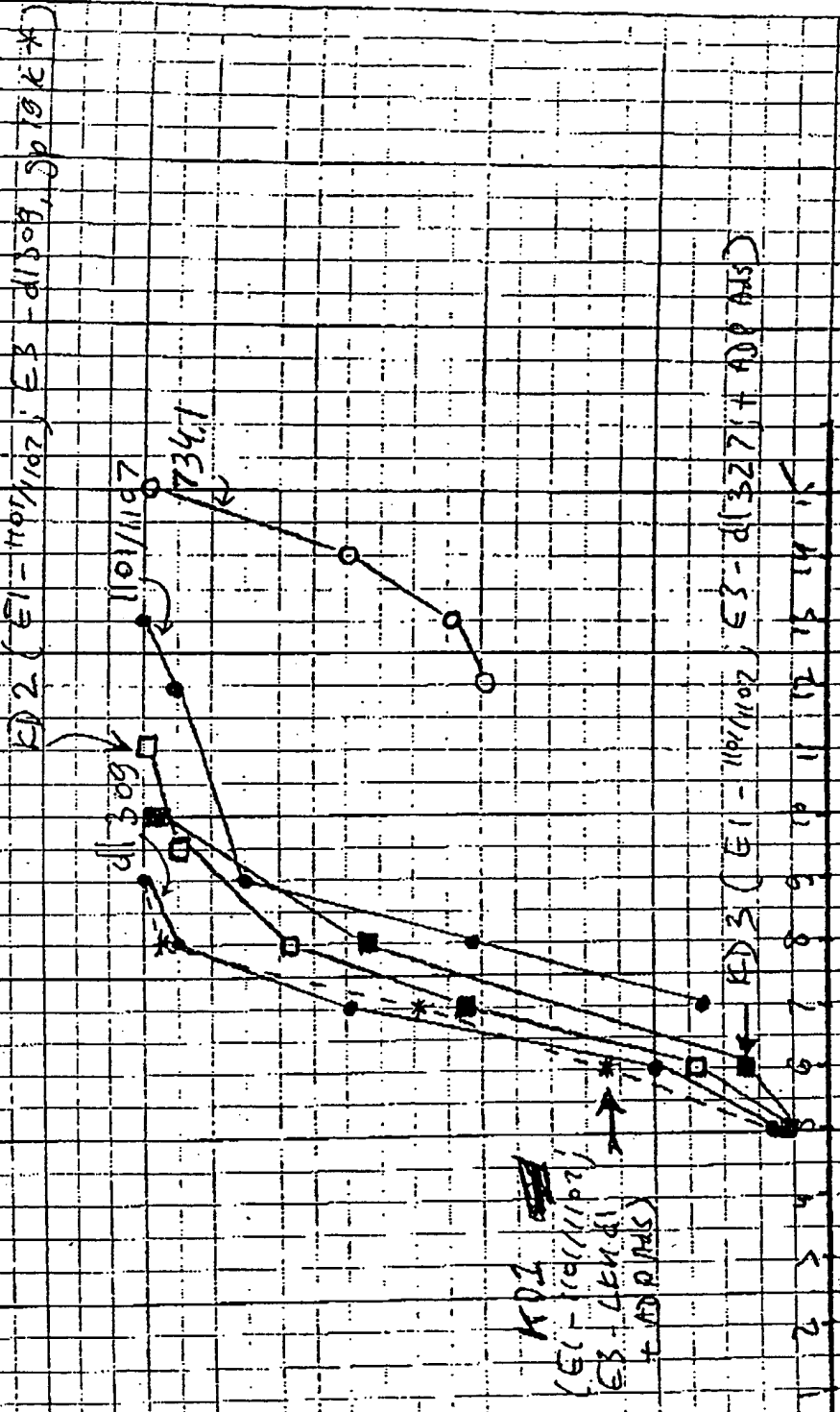
AS49 cells - in progress.

iii) Starting IP with ADP sera to check for  
ADP expression in the viruses direct.50 SHEETS  
22-141  
100 SHEETS  
22-142  
200 SHEETS  
22-144

Kostys

Plaque development assay; AS413 cells

Percent of final plaques



Days post infection

EXHIBIT

956

Kerby 5/30/97

E3 proteins

- I did an IP with NPGK 14.7B, the result is not conclusive.
- I am repeating the MTZ cloning.

Fool project

- The tested four plaques were not expressing  $\beta$ -Gal. I found two blue cells, I don't know where they came from.
- I have 3 new plaques, they came at the same time as lacZ controls.

Crm A

I am cloning CMV-CrmA in the left end plasmid

Cancer therapy

I am titrating the time course in A549, MCF-7 and 283 cells. Started yesterday.

E2-Gal

I am waiting for plaques  
No plaques on lacZ control yet either.

(24 and 8 days).

In Chip's GAL4-TAD 293 cell line

BAG1, E2A promoter substituted  
w/ Gal4/TATA.

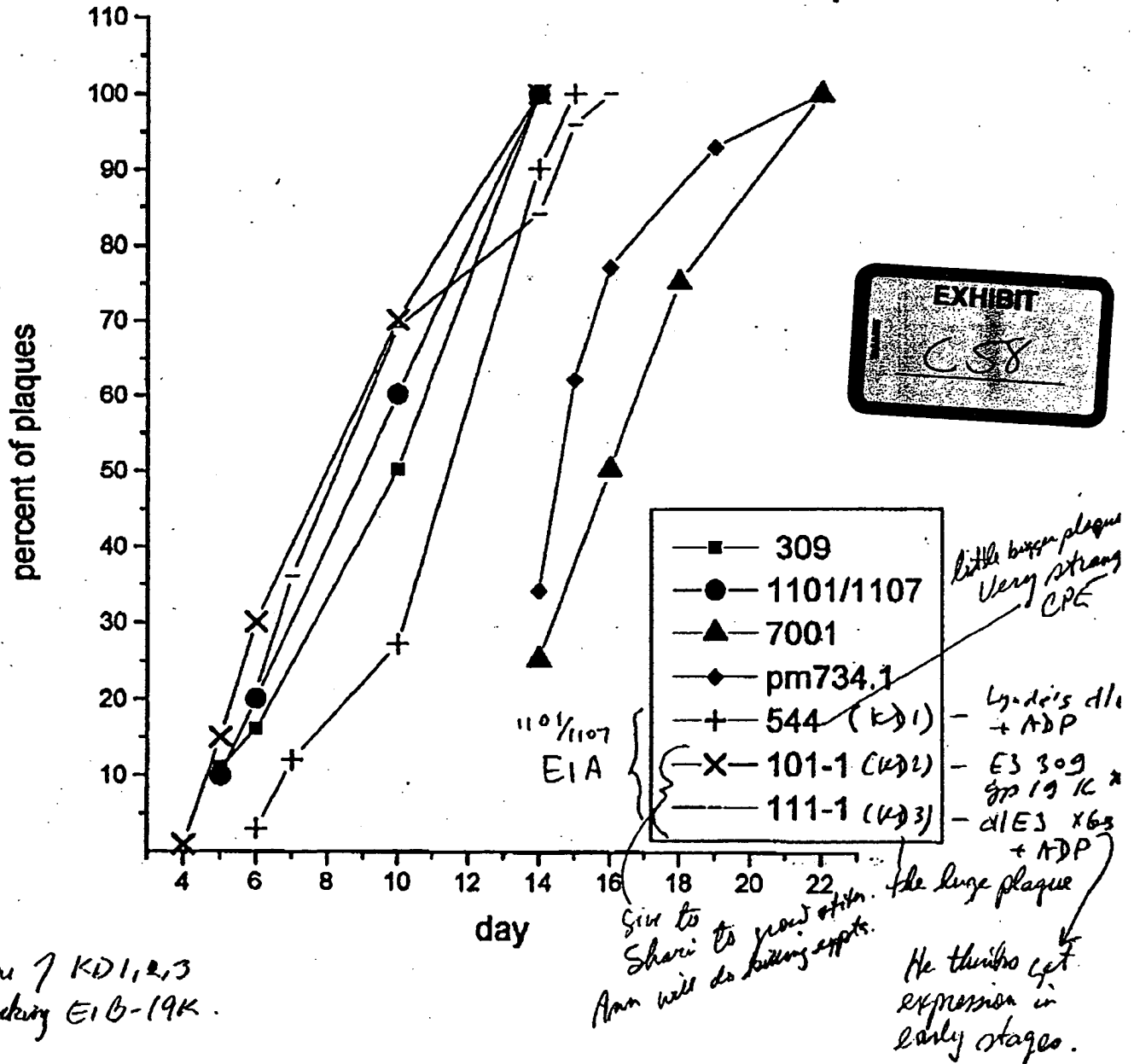
Chip - give Ad2 x Ad5 combo. in pCI, to Karl. Make Ad vector  $\pm$  lacZ  
- give R57LTR-14.7k, pCI-14.7k, pMT2-14.7k to Milan. Make Ad vector  $\pm$  lacZ  
style - will handle gp19k/CMV, Ad2 version.

EXHIBIT

C57



## Plaque development assay (293 cells)



Make on 7 KD1,2,3  
also looking E16-19K.

A529 expt - now at 14 days. Look like 293, but 309 is a bit faster.  
So 1101/1107 mutant does slow plaques a little.

Moham 5/30/97

1. C.F. Project:

- a) 293 cells (2 Rep 78), transfected by d17001 DNA (2<sup>nd</sup> started showing plaques (~40/60mm dish) ~ 15 days  
 cells co-transfected with pAE1 Sp1A/37 SpC Rep78 and d17001 + ClaI digested viral DNA - no plaques yet. (15th day)  
 cells transfected by d17001 + ClaI - no plaques -

(I am going to several transfections using d17001 + ClaI digested DNA)

- b) MCF-7 ~~cells~~ cells were transfected - as following -  
 plasmids for I.F. for Rep78  
 1) pCDNA3 Rep78 (I.F. will show Rep78 expression)  
 2) pCMVITF  
 3) pCMVITF + pAE1 Sp1A/37 SpC Rep78  
 4) pAE1 Sp1A/37 SpC

- c) Primers for Rep78 antisense → ordered.

2. EB project:

- a) viruses Ct14.7 (Pacina promoter), 14.7 LacZ (p01 2 14.7)  
 did not show plaques at 10<sup>-7</sup> dilution.

particles/OD; for wt Ad 9.04 × 10<sup>13</sup> /ml - STR.

|                         |        | 1     | 2     | ptu                       | Particle/OD = OPD        |
|-------------------------|--------|-------|-------|---------------------------|--------------------------|
| Cell                    | r700   | 0.174 | 0.167 | 9.88 × 10 <sup>10</sup>   | ~1.54 × 10 <sup>12</sup> |
|                         | Ct14.7 | 0.232 | 0.217 | ~                         | ~1.9 × 10 <sup>12</sup>  |
| CPE GSK - APGK 14.7     |        | 0.869 | 0.861 | 3 × 10 <sup>9</sup> 293   | - -                      |
| (medium is interfering) |        |       |       | 2.3 × 10 <sup>9</sup> 293 |                          |

→ Going to repeat with more dilutions.

- b) After Ct14.7 and 14.7 LacZ ptn → I.F.

EXHIBIT

C59

3. Cancer project: HEL 299 cells; 500 pfu/cell

dl 309 growth arrested cells → stained  
→ plaques start~~ing~~ appearing. Count on 2 day

EXHIBIT

C60

Sheet1 Chart 6

Growth curve 500 pfu/cell  
(HEL 299 cells)

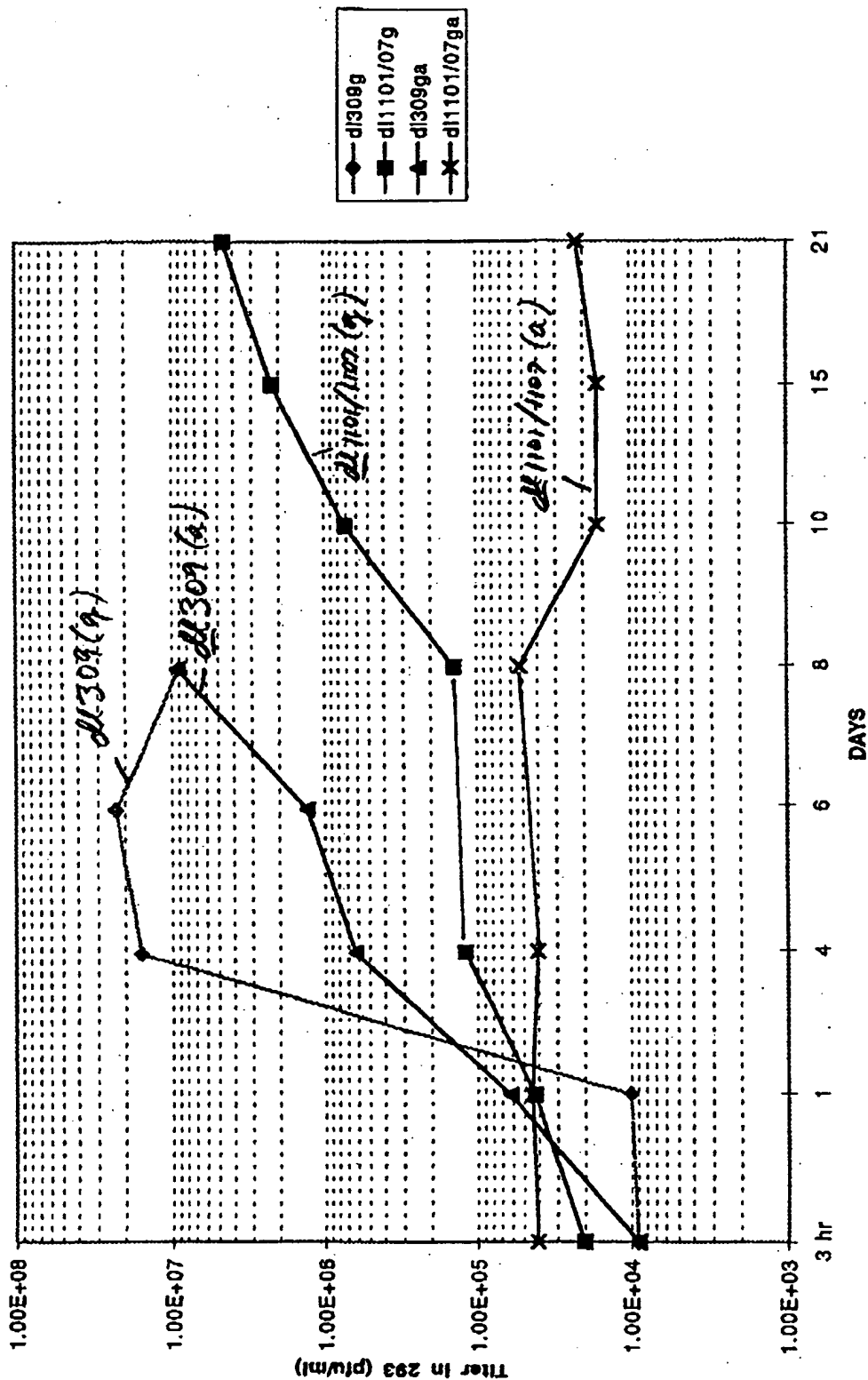
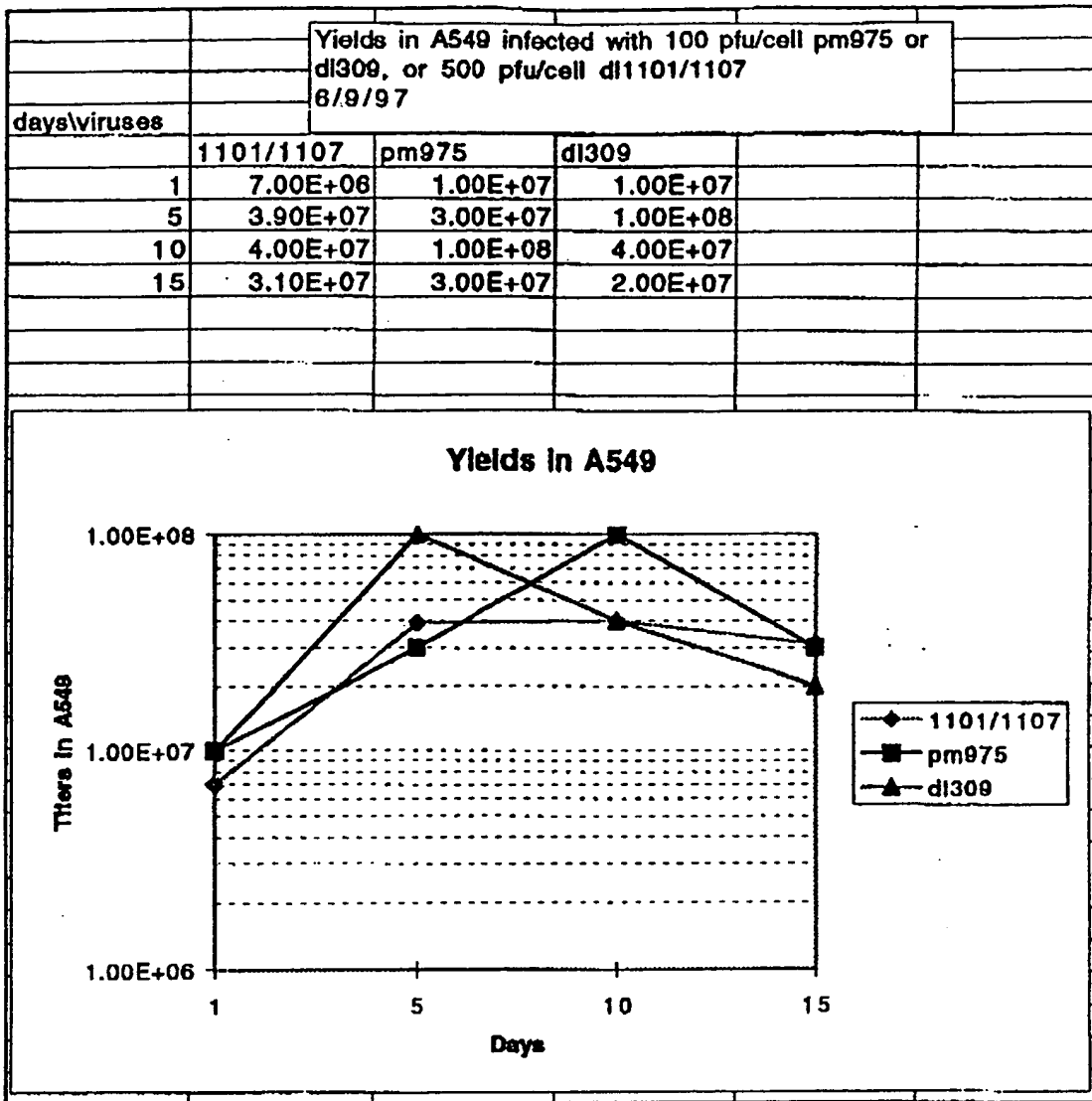


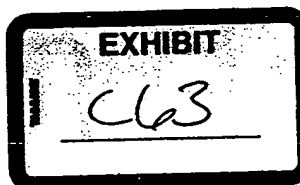
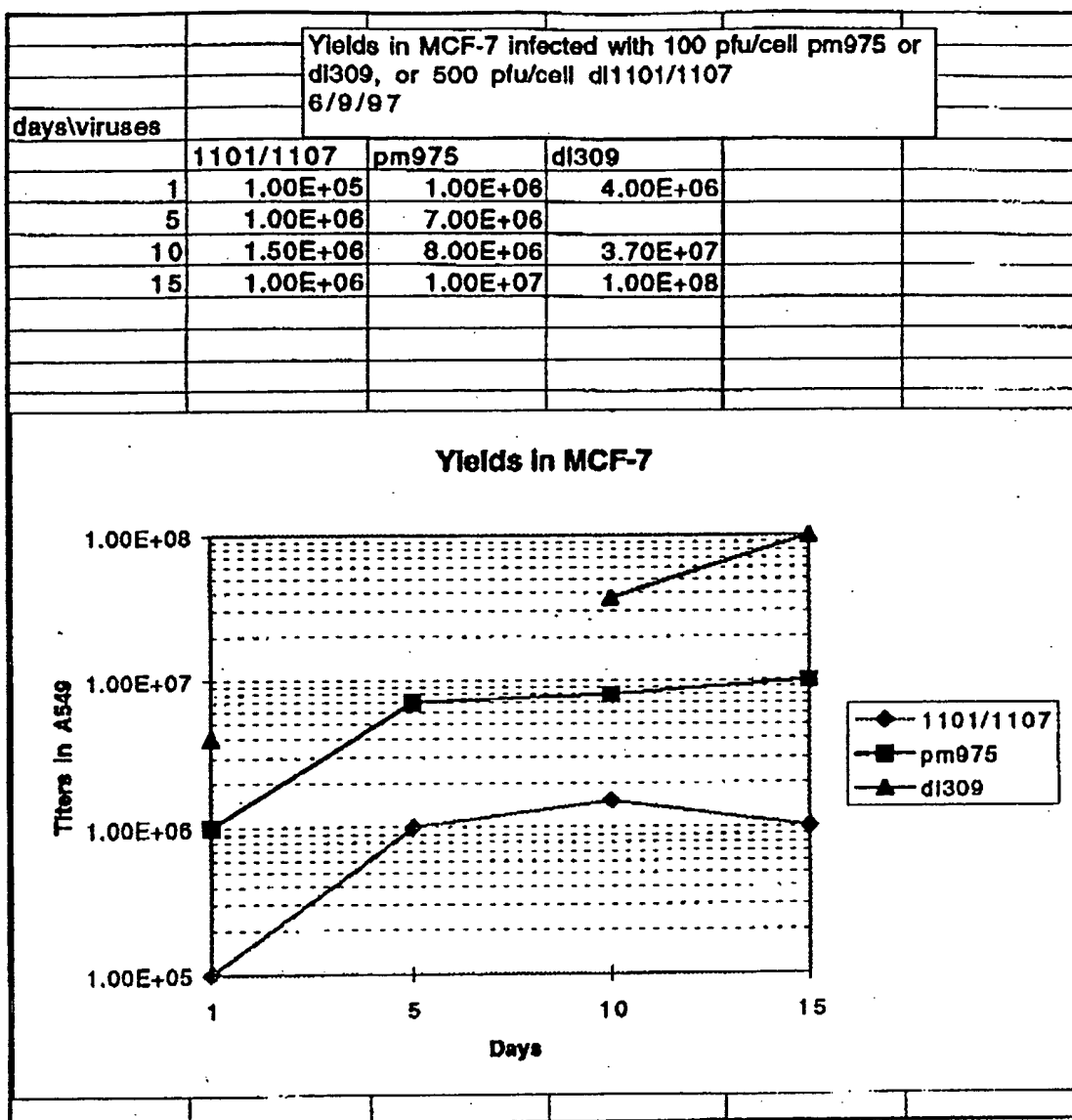
EXHIBIT  
C61

Figure 1.

## Sheet1

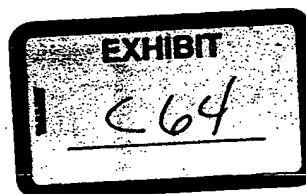
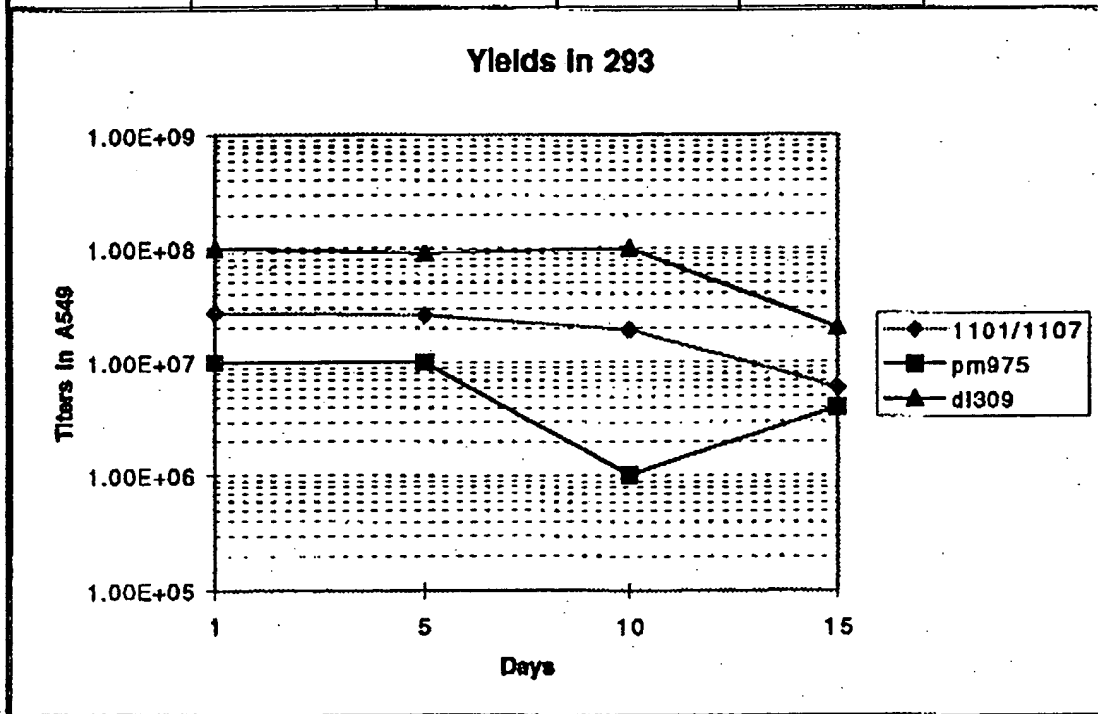


## Sheet1



## Sheet1

| Yields in 293 infected with 100 pfu/cell pm975 or dl309, or 500 pfu/cell dl1101/1107<br>6/9/97 |           |          |          |
|--|-----------|----------|----------|
| days\viruses   | 1101/1107 | pm975    | dl309    |
| 1  | 2.70E+07  | 1.00E+07 | 1.00E+08 |
| 5  | 2.80E+07  | 1.00E+07 | 9.00E+07 |
| 10   | 1.90E+07  | 1.00E+06 | 1.00E+08 |
| 15   | 6.00E+06  | 4.00E+06 | 2.00E+07 |



6/15/97 NOS 1/2

1) E3 project

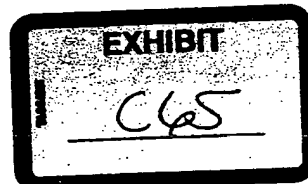
Made the plasmid with E3 (SrfI-NdeI) from pm754.1 (ADP-) in pCDNA3.1zeo(+). Gave maxiprep to Karol to check for E3 proteins.

2) Cancer therapy project

i) There are no plaques yet in dishes with p152 (E4SPB) transfection (2 weeks).

ii) Plaque development assay for E3 ADP viruses in A549 - see graph.

iii) IP with anti-ADP serum didn't work (even in positive control). Next week going to try IP with controls and various sera to optimize experiment conditions.





Plaque development assay, A549

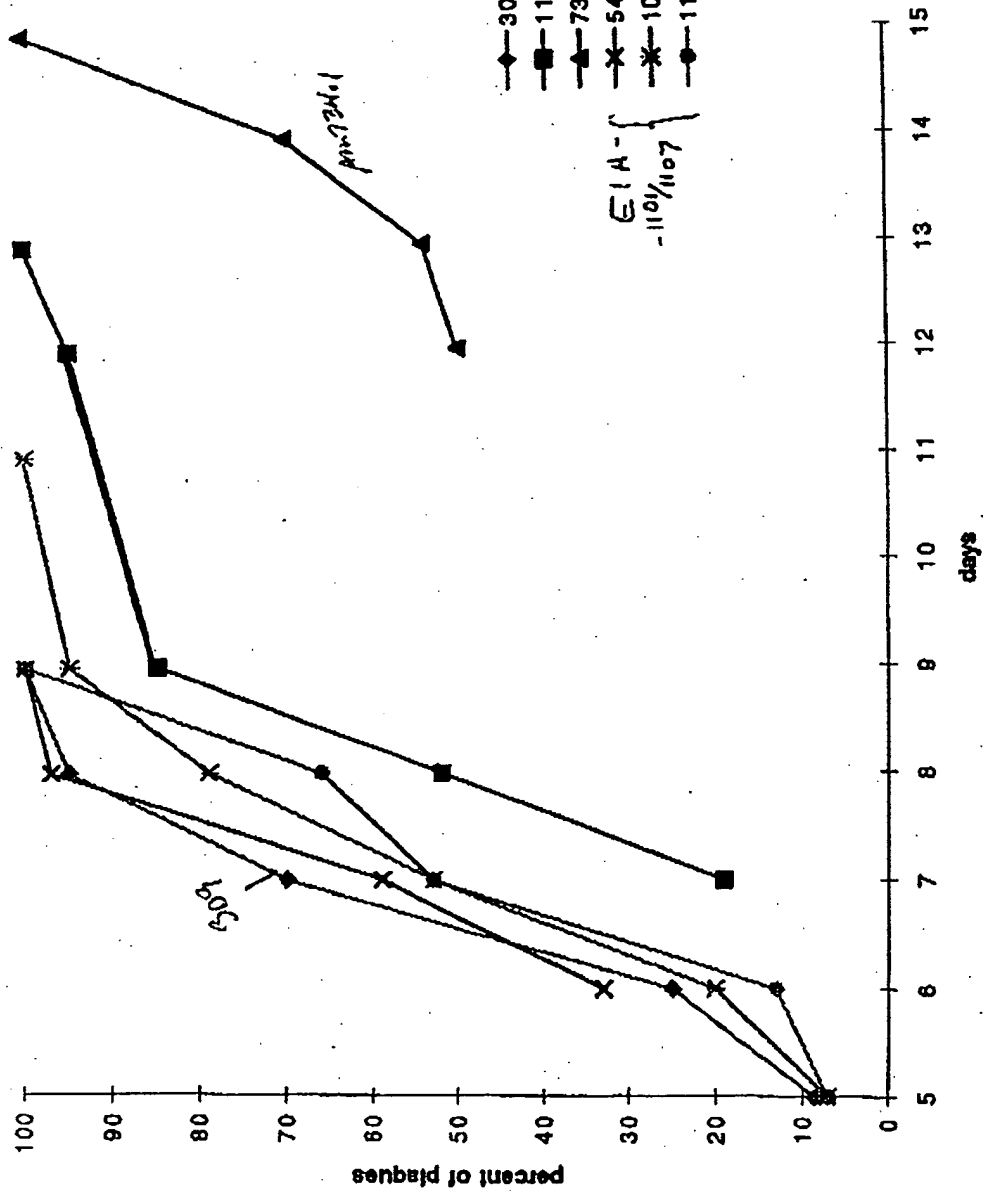


EXHIBIT  
C66

Mohan, 6/13/77

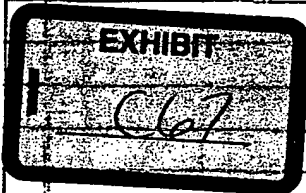
1. C.F. project

- a) 293 cells expressing anti. by p2E1Sp1A/3.7Sp1 Rep78 a viral DNA - shows the f slow.
- b) 293 < Rep78 cell lines sho. fragment by PCR.

2. E3 project:

a)

Particles/OD, OPU



|          | OPU <sup>1</sup>      | OPU <sup>2</sup>     | Pfu                   |
|----------|-----------------------|----------------------|-----------------------|
| r700     | $1.52 \times 10^{12}$ | $9.8 \times 10^{11}$ | $9.88 \times 10^{10}$ |
| C+14.7   | $1.9 \times 10^{12}$  | $9 \times 10^{11}$   |                       |
| GP19K    |                       | $2.8 \times 10^{11}$ | $1.2 \times 10^{10}$  |
| ITR LacZ |                       | $2.2 \times 10^{12}$ | $9.8 \times 10^{10}$  |

Pfu will be  $\approx 10^7$  replicable

- b) Lact purified r700, C+14.7, and GP19K virus - 100  $\mu$ l  $\rightarrow$  viral DNA  $\rightarrow$  gel.

It seems from the gel, OPU/DNA ratio looks reasonable. ~~rr~~

If it is true then virus prepn - does it have more defective particles in C+14.7, or toxic?

- c) I.F: A549 cells, r700, NP6-K 14.7 and GP19K (50 p.p.m. < 14.7.

- d) Transferring GP19K fragment from pCI19K to left hand plasmid p2E1Sp1A and p230 (p2E1Sp1/PA1) #30 NP6K qv9  $\rightarrow$  pCI. Have done p2E1Sp1 AT (LacZ opposite end). I have a clone in p2E1Sp1A  $\rightarrow$  plasmid prepn  $\rightarrow$  I have clones in p230 vectors  $\rightarrow$  analysing. PA1, PA2, LacZ

3. Cancer project: MEL 293 cells, dl309 and dl1101 (LacZ) (50 p.p.m.) See growth curve graph

Sheet Chart 6

Growth curve 500 pfu/cell

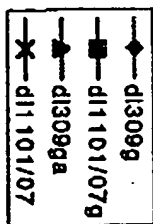
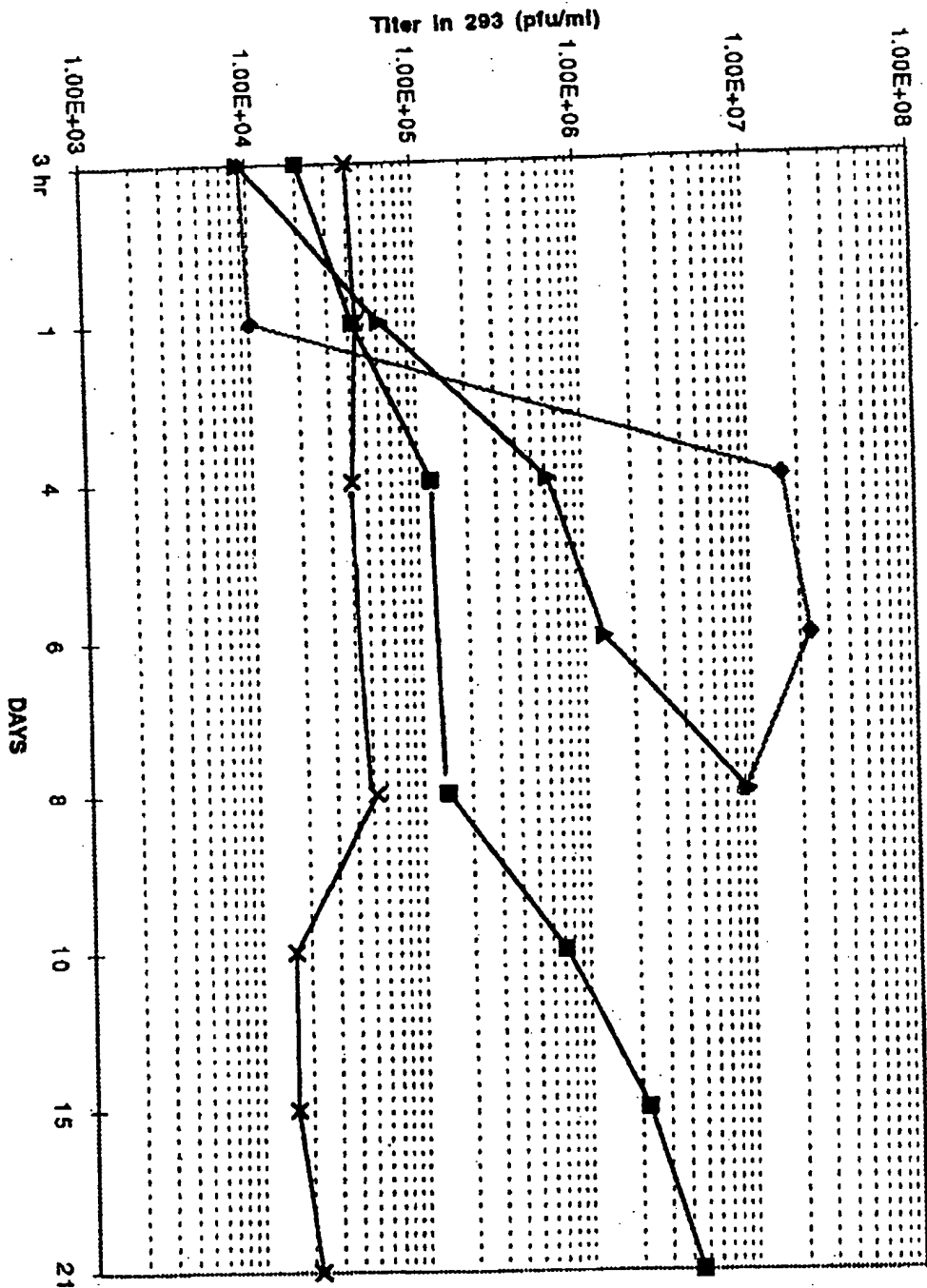


EXHIBIT  
C68

Kasov 6/12/3+

E3 proteins

- I did a Western with Ct 14.7 and VP6k147B
- I am doing an IF with Kostya's CMV E3 and Todor's 760 R10
- I have a problem with cloning, I am working on it.

Fas L project

- I have ~ 15 new plaques, they came at about the same time as 12c2. I am growing them up.

Cancer therapy

- Time course in 293, A549 and MCF 7 is done, I am making 0 hour timepoints.

E2-Gal

- No plaques yet (not even in control).

22-141 50 SHEETS  
22-142 100 SHEETS  
22-144 200 SHEETS

Kostya 6/11/97

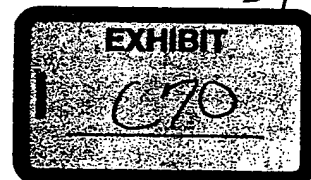
# 1) E3 project.

734.1 ← E3

i) Have made the left-hand plasmid based on p#30 (pΔE1sp + lacz-RSV) with CMV-E3 pm734.1 expression cassette in it. Now making cell prep. to check for expression and making virus.

ii) Failed to clone some CMV-E3 pm734.1 into plasmid with Lynda's E3 deletion. Going to reclone CMV-E3 pm734.1 into pCI 2Xβe (Lynda), then I'll be able to put the expression cassette to PciI site of either Lynda's plasmid or pBK611.

iii) Karol has shown that Terry's PC1/14.7 either don't express or express weak. I'm going to reclone 14.7 from pMT2/14.7 to pCDNA3.1(zeo(+)), then CMV-14.7 to p#30.



# 2) Cancer therapy project.

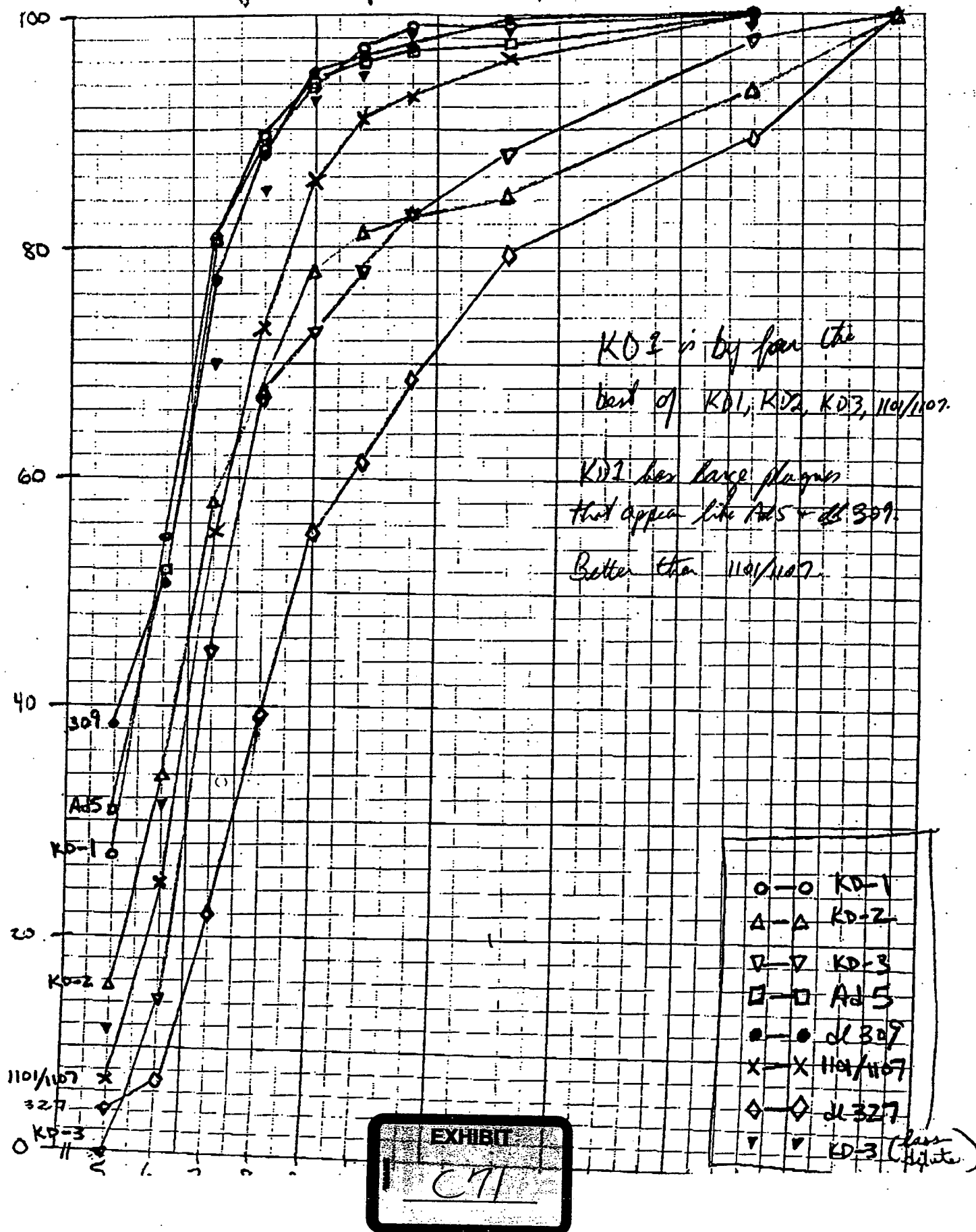
i) Got 293/E4 from Valery Krongliak. Now growing them up to freeze and to repeat transfection experiments with SP-B ↔ E4 promoter substitution.

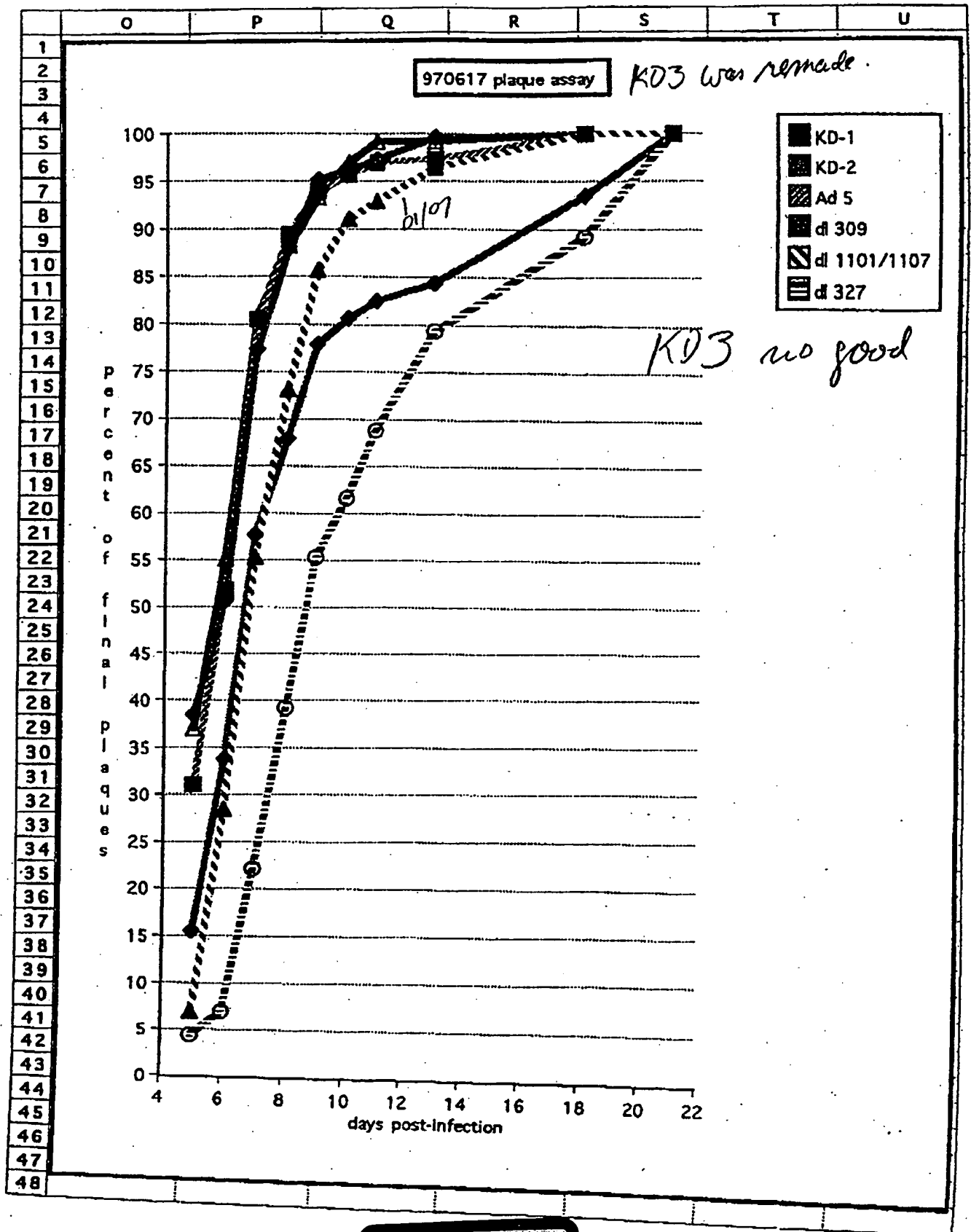
- Plaques are later than E4(+)
- Plaques are of different morphology (d1366 for me as well)
- Defect of linearization?

ii) 293 cotransfection of p626 E4 ↔ CMV + d1366.  
293 cotransfection with E4 expressing plasmids.

iii) Ann's results with K13

## 970617 Plaque Assays on AS49





EXHIBIT

C72

|    | A           | B    | C    | D    | E    | F    | G    |
|----|-------------|------|------|------|------|------|------|
| 1  | 970617 PA   |      |      |      |      |      |      |
| 2  | KD-1        | 5    | 6    | 7    | 8    | 9    | 10   |
| 3  |             | 37   | 55.2 | 80.9 | 88.4 | 93.4 | 97.1 |
| 4  | KD-2        | 5    | 6    | 7    | 8    | 9    | 10   |
| 5  |             | 15.6 | 33.9 | 57.8 | 67.9 | 78   | 80.7 |
| 6  | Ad 5        | 5    | 6    | 7    | 8    | 9    | 10   |
| 7  |             | 31.2 | 51.9 | 80.6 | 89.5 | 94.1 | 95.8 |
| 8  | d 309       | 5    | 6    | 7    | 8    | 9    | 10   |
| 9  |             | 38.5 | 50.8 | 77.5 | 88.1 | 95.1 | 96.3 |
| 10 | d 1101/1107 | 5    | 6    | 7    | 8    | 9    | 10   |
| 11 |             | 7.1  | 28.6 | 55.4 | 73.2 | 85.7 | 91.1 |
| 12 | d 327       | 5    | 6    | 7    | 8    | 9    | 10   |
| 13 |             | 4.5  | 7.1  | 22.3 | 39.3 | 55.4 | 61.6 |
| 14 |             |      |      |      |      |      |      |
| 15 |             |      |      |      |      |      |      |
| 16 |             |      |      |      |      |      |      |
| 17 |             |      |      |      |      |      |      |
| 18 |             |      |      |      |      |      |      |
| 19 |             |      |      |      |      |      |      |
| 20 |             |      |      |      |      |      |      |
| 21 |             |      |      |      |      |      |      |
| 22 |             |      |      |      |      |      |      |
| 23 |             |      |      |      |      |      |      |
| 24 |             |      |      |      |      |      |      |
| 25 |             |      |      |      |      |      |      |
| 26 |             |      |      |      |      |      |      |
| 27 |             |      |      |      |      |      |      |
| 28 |             |      |      |      |      |      |      |
| 29 |             |      |      |      |      |      |      |
| 30 |             |      |      |      |      |      |      |
| 31 |             |      |      |      |      |      |      |
| 32 |             |      |      |      |      |      |      |
| 33 |             |      |      |      |      |      |      |
| 34 |             |      |      |      |      |      |      |
| 35 |             |      |      |      |      |      |      |
| 36 |             |      |      |      |      |      |      |
| 37 |             |      |      |      |      |      |      |
| 38 |             |      |      |      |      |      |      |
| 39 |             |      |      |      |      |      |      |
| 40 |             |      |      |      |      |      |      |
| 41 |             |      |      |      |      |      |      |
| 42 |             |      |      |      |      |      |      |
| 43 |             |      |      |      |      |      |      |
| 44 |             |      |      |      |      |      |      |
| 45 |             |      |      |      |      |      |      |
| 46 |             |      |      |      |      |      |      |
| 47 |             |      |      |      |      |      |      |
| 48 |             |      |      |      |      |      |      |

EXHIBIT

C73



|    | H    | I    | J    | K   | L | M | N |
|----|------|------|------|-----|---|---|---|
| 1  |      |      |      |     |   |   |   |
| 2  | 11   | 13   | 18   | 21  |   |   |   |
| 3  | 99.2 | 99.2 | 100  | 100 |   |   |   |
| 4  | 11   | 13   | 18   | 21  |   |   |   |
| 5  | 82.6 | 84.4 | 93.6 | 100 |   |   |   |
| 6  | 11   | 13   | 18   | 21  |   |   |   |
| 7  | 97   | 97.5 | 100  | 100 |   |   |   |
| 8  | 11   | 13   | 18   | 21  |   |   |   |
| 9  | 97.5 | 99.6 | 100  | 100 |   |   |   |
| 10 | 11   | 13   | 18   | 21  |   |   |   |
| 11 | 92.9 | 96.4 | 100  | 100 |   |   |   |
| 12 | 11   | 13   | 18   | 21  |   |   |   |
| 13 | 68.8 | 79.5 | 89.3 | 100 |   |   |   |
| 14 |      |      |      |     |   |   |   |
| 15 |      |      |      |     |   |   |   |
| 16 |      |      |      |     |   |   |   |
| 17 |      |      |      |     |   |   |   |
| 18 |      |      |      |     |   |   |   |
| 19 |      |      |      |     |   |   |   |
| 20 |      |      |      |     |   |   |   |
| 21 |      |      |      |     |   |   |   |
| 22 |      |      |      |     |   |   |   |
| 23 |      |      |      |     |   |   |   |
| 24 |      |      |      |     |   |   |   |
| 25 |      |      |      |     |   |   |   |
| 26 |      |      |      |     |   |   |   |
| 27 |      |      |      |     |   |   |   |
| 28 |      |      |      |     |   |   |   |
| 29 |      |      |      |     |   |   |   |
| 30 |      |      |      |     |   |   |   |
| 31 |      |      |      |     |   |   |   |
| 32 |      |      |      |     |   |   |   |
| 33 |      |      |      |     |   |   |   |
| 34 |      |      |      |     |   |   |   |
| 35 |      |      |      |     |   |   |   |
| 36 |      |      |      |     |   |   |   |
| 37 |      |      |      |     |   |   |   |
| 38 |      |      |      |     |   |   |   |
| 39 |      |      |      |     |   |   |   |
| 40 |      |      |      |     |   |   |   |
| 41 |      |      |      |     |   |   |   |
| 42 |      |      |      |     |   |   |   |
| 43 |      |      |      |     |   |   |   |
| 44 |      |      |      |     |   |   |   |
| 45 |      |      |      |     |   |   |   |
| 46 |      |      |      |     |   |   |   |
| 47 |      |      |      |     |   |   |   |
| 48 |      |      |      |     |   |   |   |



06/20/97

Hostya

1) E3 project

i) Started cloning of CMV-E3 pm 734.1 expression cassette to left-hand plasmid (p#30) and into E3 (in place of Lyda's E3 deletion).   
 → Clone into SPC expression cassette to TC minimal.

ii) Started cloning of CMV-14.7 expression cassette from pc1-14.7 (Terry-Chip) to left-hand plasmid.

2) Cancer therapy project

Have no problems with SPB ↔ E4 promoter substitution. (293-TT1Mdan)

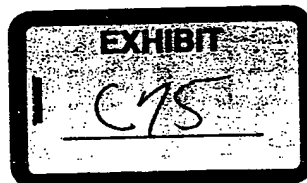
I'm starting with Hep/E4, A549/E4 from GTI, need to check them for ability to support growth of 366 (E4d1).

W162 - (expressing E4) - if Ann can find them.

Growing up plasmids containing E4 under the control of CMV and native E4 promoters. (for cotransfection)

Valeri Krongliak wrote me that he would be able to send us 293/E4 (VK2-20) when Dr. Wold write him a letter with request. His address is on the next page.

TI-E4 deletion cassette.





Valeri Krougliak,  
Assistant Professor  
Institute for Gene Therapy  
Mount Sinai Medical Center, box 1496,  
One Gustave L. Levy Place  
New York, NY, 10029  
Tel. 212-824-7748  
Fax: 212-849-2437  
E.mail krougv01@doc.mssm.edu

293 expressing EU (VK2-20)

Published in Human Gene Therapy 6: 1575-1586  
(December 1995)

Have 3 plasmids with ~~et~~ deletion.  
Cannot rescue virus.

EXHIBIT

C77

6/27/97

Kosiyu

1) E3 project

i) Cloning of CMV-E3 pm 734.1 expression cassette into left-hand plasmid and into plasmid with E3 deletion is in progress.

ii) Terry's pCI-14.7. Made miniprep of the plasmid, it looks like this is pCI with Ad5 14.7 in it. Made maxiprep, Karl is checking it for expression. Now doing recloning of CMV-14.7 expression cassette from this plasmid to left-hand plasmid.

2) Cancer therapy project

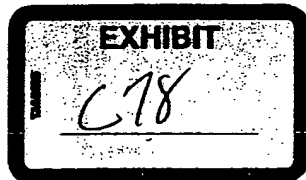
Got A549/E4; Hep/E4; W162 from Ann. Doing plaque assay of dl366 on these cells with 293 and A549 as controls.

Going to try to optimize transfection efficiency for these cells.

Going to try transfection-infection experiment (infection with 366, transfection with CMV-E4, SPB-E4 & E4-Epromoter as a control) on 293 cells.

Going to try cotransfect 293 with 1101/1107/E4R1 + CMV-E4 plasmid).

293/E4 (Valeri Krouglick)?



PINE 3.95 MESSAGE TEXT

Folder: MAIL Message 41 of 42 90%

Date: Mon, 23 Jun 1997 17:28:35 -0600 (CST)  
From: WOLDWS@SLU.EDU  
To: "KROUGV01@DOC.MSSM.EDU"@SLU.EDU  
Cc: WOLDWS@SLU.EDU  
Subject: Request for cells

Dear Valeri,

I hope all is going well with you. I'm happy to see that you have a faculty position in a good university.

I am writing to request the 293 cell line expressing E4 (VK2-20) that you published in Human Gene Therapy (6:1575-1586, 1995). We have several plasmids with deletions in E4, and we have been unsuccessful in rescuing them into virus. We would use your cell line for that purpose.

Thank you in advance.

Sincerely,

|              |             |           |              |            |           |
|--------------|-------------|-----------|--------------|------------|-----------|
| ? Help       | M Main Menu | P PrevMsg | - PrevPage   | D Delete   | R Reply   |
| O OTHER CMDS | V ViewAttch | N NextMsg | SpC NextPage | U Undelete | F Forward |

EXHIBIT

C79

970687

Gene Therapy Meeting

①

Fast Ad

Karl

13 of Fast vector ~ 11 blue (23 plaques total)  
1 blue + killed MCF7 - CrmA

3 plaques by PCR do have Fast gene.

PCR - Fast in Nipples left and shoulder. pBHG 11

Next

5 from 60 mm dishes

Check by IF - all plaques.

Check by PCR for Fast.



②

Ad-14.7 PGK vector - expression at 14.7 d pi  
p734.1 - GFP19 exp., 14.7k exp.

③

C<sup>+</sup> kills cells at 2 days pi. C50 stock - strange discrepancy between particles & titers.

Raffinose → supernatant → perhaps some expression.

This plasmid expresses β-gal in transient COS cells.

β-actin promoter & out enhancer; pol II for 14.7k.

This was done at 10<sup>8</sup> PFU (titer could be much higher)

Repeat using titer calculated from OD & DMSO assay.

This was better than β-actin promoter enhancer in COS.

Karl tried to make vector from several diff promoters/polys;  
this was only one that gave plaques.

22-141 50 SHEETS  
22-142 100 SHEETS  
22-144 200 SHEETS

Dr. G. L. D. Silva



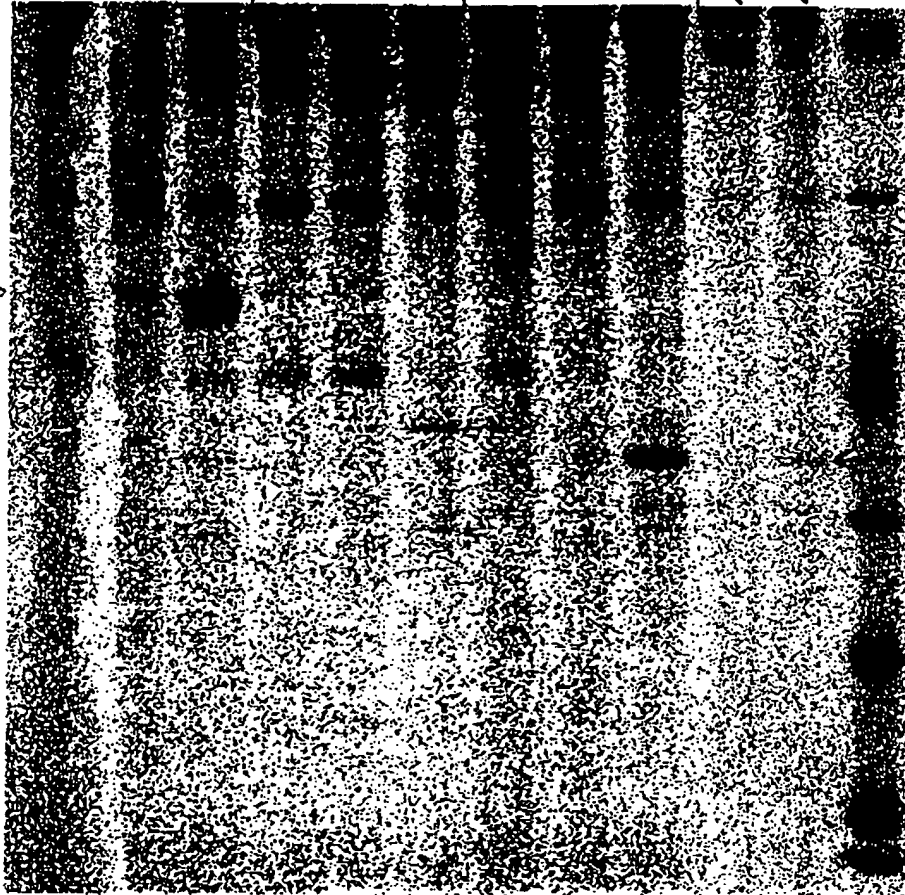
GEL 1997:06:27 12:46:35, Range - 0.01-249.86 Counts, 1.00x

Karl

Transfection of p734.1 into 293  
exposed overnight on  
PhosphorImager.

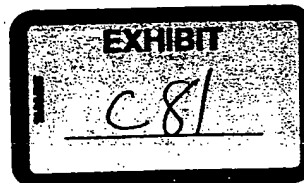
| 293 | 293 + 734.1 | 700 (200 pi) | 293 | 734.1 | 700 (200 pi) | 293 | 734.1 | 700 (200 pi) | AS219 | AS219 + NPK-14.7B<br>day 3 pi |
|-----|-------------|--------------|-----|-------|--------------|-----|-------|--------------|-------|-------------------------------|
|     |             |              |     |       |              |     |       |              |       |                               |

9p19K →



14.7K  
is mod

|         |   |   |   |   |   |   |   |   |
|---------|---|---|---|---|---|---|---|---|
| α 9p19K | + | + | + |   |   |   |   |   |
| α R1Dβ  |   |   |   | + | + | + |   |   |
| α 14.7  |   |   |   |   |   |   | + | + |





Mohan, 6/27/97

1 C.F. project:

- a) 4 plaques (SPC Rep78 virus?) <sup>(may get CPE)</sup> are growing in 293 78 cells. 3 plaques showed CPE. Growth is slow ~ 100% confluent.
- b) RT-PCR for Rep78 antisense RNA were done employing RNA + Reverse transcriptase to verify the PCR fragment is due to RNA and not from DNA contamination. It seems 293/Rep78 cell line is expressing Rep78 antisense RNA.
- c) pSub VIII plasmid → chips → plasmid preparation. Chip with mds.

2 E3 project:

|       | Particles/OD           | OD                    |
|-------|------------------------|-----------------------|
| a)    | 9 got six viruses from |                       |
|       | pfu/ml                 | OD                    |
|       | $2.4 \times 10^{11}$   | $0.1$                 |
| d1722 | $1.35 \times 10^{11}$  | $0.99 \times 10^{12}$ |
| d1731 | $1.44 \times 10^{11}$  | $1 \times 10^{12}$    |
| d1739 | $7.7 \times 10^{10}$   | $1.72 \times 10^{12}$ |
| d1753 | $1.07 \times 10^{11}$  | $1.18 \times 10^{12}$ |
| d1762 | $1.58 \times 10^{11}$  | $3.18 \times 10^{12}$ |

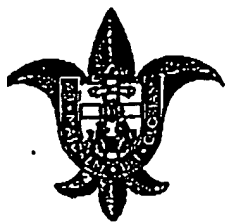
d1704 OD is lesser than pfu. (Repeat)  
 - do  $10^8$  pfu +  $10^9$  pfu

- b) MCF-7 cells transfected by p2E1SPK/gp12K and p30 (Kimpens) gp12K (\*2, \*3 & \*) got contaminated. (Yeast) Repeating again for I.F.
- c) Cloning Ad2, Ad5 RID into p2E1SPK and p30 is in progress (from Karl)
- d) KTI (14.7 LacZ) gave  $6.2 \times 10^6$  pfu → More CPE  
 Stocks → 5ml each - 1.1

EXHIBIT

C82

## **EXHIBIT D**



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**SAINT LOUIS  
UNIVERSITY**

Department of Molecular  
Microbiology & Immunology

Health Sciences Center  
School of Medicine

**Facsimile Transmittal**

**Date:** 12-19-02

**From:** William Weld

**Phone:** 314-577-8432

**Fax:** 314-773-3403

**Deliver to:** Dan Kaster

**Phone:** \_\_\_\_\_ **Fax:** 552-7305

**Total number of pages including this page:** 15

**Message:**

8 - 100MM

7/13

todo

phone:

Ab in white/red File on Arms desk

Inventory virus book \* label / + verify

✓ (KBS from freezer) / when brought up (7001)

Develop the gel (Monday)

$2 \times 10^5 / \text{ml}$

$7.5 \times 10^7$

✓ (cells frozen + thrown out) ✓

no less than 200  
3mls / 200mls cell

(leave sitting / media)

Ad 1 + Ad 6 Virus stock (5mls of stock)

Set up 1-100ml → 800

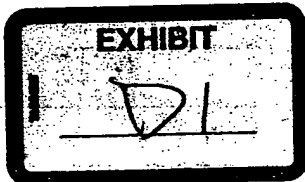
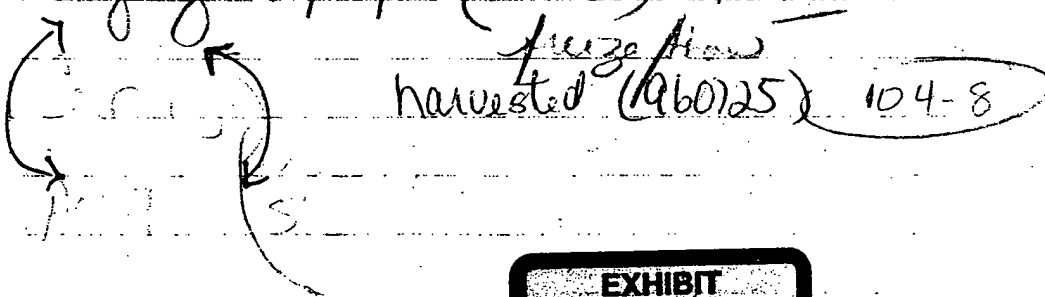
grow up 11.6 pre-absorbed. ✓ Set up \*

293 cells from Kaye (Duke Univ) OK  
Ginsberg A549 III (earliest stock) (175 flasks)

V9103 5-2-1 stock 50ml → 100mm plate → flasks

V9103 5-4-1 spin down / media 20% FBS 100ml → 100mm plate  
(harvested) (960705)

Bayley 11/07/6 (911003) 104-8 loc 50ml → 100mm plate



9/4/96

CSD Banded the virus Adl 960825  
1101/1107 Baileys (960802)

- 1 freeze (thaw)
- 2 sonicate X 2
- 3 Vol after son 1101 < 2mls

Adl < 19mls

add  $V \times 0.51 = g$  of GCL

10.71g in 1101/1107 Baileys  
9.69g in Adl.

infect 3 liter spinner with  
dl 717 Vp 891208 Titer  $1.3 \times 10^{11}$

cell count 3 liter  $3.5 \times 10^5$   
Vol = 3 liters (3000mls)

$3.5 \times 10^5 \times 3000\text{mls} = 10.5 \times 10^8$  total  
cells

use 20 pfu's / cell

$\frac{162\mu\text{l} : \text{use}}{1.3 \times 10^{11}} \times 20 \text{ pfu's}$

KB's are healthy no clumping.

EXHIBIT

D2

9/17

①

1. Atlanta 6013E  
 $\frac{19}{4} \times 3 =$  first (cells are shriveled up)  
 $1.4 \times 10^5$

\* 2. Brahitakee  
 6m1228  
 $\frac{28}{4} \times 3 =$  cells look very healthy  
 $2.1 \times 10^5$

3. NYC Apa 4819  
 $\frac{21}{4} \times 3 =$  nice + healthy cells very full + round  
 $2.3 \times 10^5$

4. present 4m1938  
 $\frac{21}{4} \times 3$  cells are dividing now  
 smaller cells but healthy  
 $1.5 \times 10^5$

5. NYC AF B4870  
 $\frac{22}{4} \times 3$  cells look great  
 $1.6 \times 10^5$

6. Biow 6m0741  
 $\frac{21}{4} \times 3$  strange shape of cells  
 Rough edges not round  
 $1.5 \times 10^5$

infected 3 liter spinner with  
 1101 / 1102 20mls  
 $3.2 \times 10^5$  cells/ml

EXHIBIT

D3

September 18, 1996.

Cloning *EcoRI*-*XbaI* B fragment to pBSSK(+)/*EcoRI*+*XbaI*  
of pFG140



Lanes: uniprep of

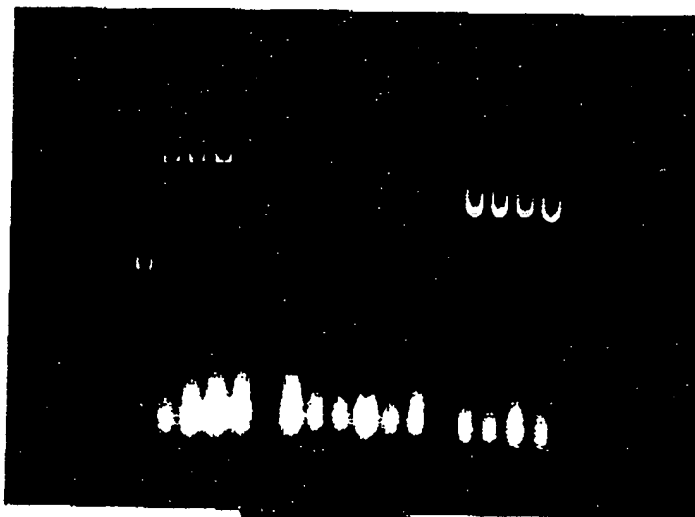
1) pBSSK(+)

2) - 9) clones

10) - 12) p21 (Mippen's plasmid)

14) - 17) pISP-C3.7.

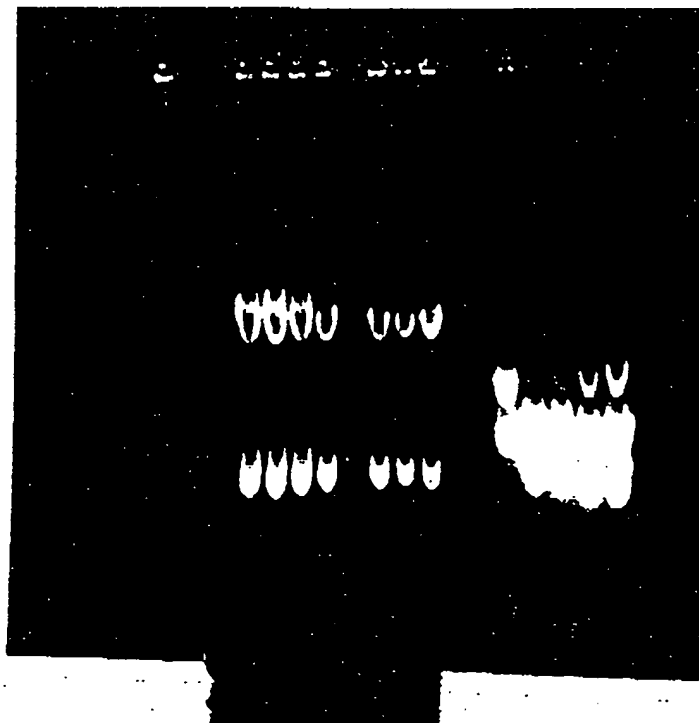
22-141 50 SHEETS  
22-142 100 SHEETS  
22-144 200 SHEETS



First experiment  
09/18/96

Restriction patterns of  
the plasmids.

- 1) Ladder
- 2) vector (pBSSK(+)) *R1*/*HaeI*
- 3) fragment B pFG140/*R1*/*XbaI*
- 4) - 11) - *R1*+*XbaI* clones 1-8
- 13), 14) - p21 (Mippen) *EcoRI*
- 15) - 18) - pSAC *HaeI*+*EcoRI*



EXHIBIT

D4

pBSKS(+) + 8 hr. nt pFG/40 RI/100

(PK1)

Lanes : 1) Ladder

2) RI + NCI

3) Bst 1107 I

4) Sun I

5) Bst 1107 I + Sun I

multicore

⇒ size of RI - vde fragment?

⇒ RNA masked 600 bp ~~RI~~

Bst 1107 I - Sun I fragment

2% agarose gel

1) Ladder

2) PK1 / Sun I + Bst 1107 I  
+ RNase

⇒ ~600 bp fragment

EXHIBIT

DS



Checking the restriction  
pattern of plasmids

in ladder

1) L 1 / Mfe + R1

3) L 1 / Xba

4) L 2 / Mfe + R1

5) L 2 / Pci

6) L 3 / Mfe + R1

7) L 3 / Pci

EXHIBIT

1 D6

28 September 1991.

minipreps.

1) Ladder incl.  
 2, 3, 4) - pLSP-B 2.2  
 5, 6, 7) - L2  $\Rightarrow$   
 $\Rightarrow$  pLKHE2A + Bam + end + Cla

8, 9, 10) - L3  $\Rightarrow$   
 $\Rightarrow$  PCR(LKH dP) Bam  $\rightarrow$  end E2A +

SPB experiment  
 10/27/96

minipreps.

1) Ladder  
 2) - 3) - L1  $\Rightarrow$   
 $\Rightarrow$  pTZAd - Xba

22-141 50 SHEETS  
 22-142 100 SHEETS



EXHIBIT

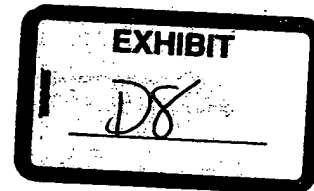
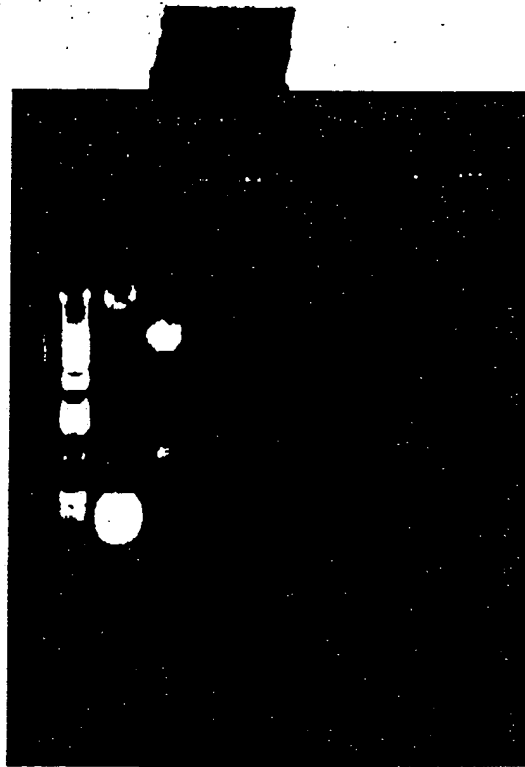
D7

September 30, 86.

Cloning - of CMV promoter into  
Bst 1107I site of p21 (Kip1).

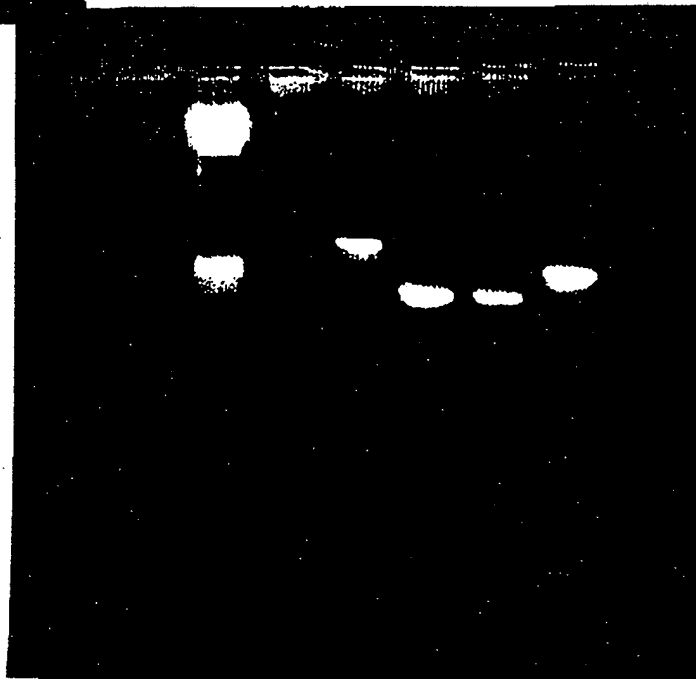
- 1) ladder
- 2) p21 / Bst 1107I
- 3) pCDNA3 / Bgl II + Bam HI

23-141 50 SHEETS  
22-142 100 SHEETS  
22-144 200 SHEETS



October 2, 86

Testing primers in PCR



- 1) ladder
- 2) KJ2 - KJ3
- 3) KJ1 - KJ2
- 4) KJ4 - KJ5
- 5) KJ6 - KJ7
- 6) KJ8 - KJ9

all 9 primers  
are OK.

October 9, 96

Analysis of clones

1-2, 4-8 of

pK1 / bst11021 + 2.1 - CIP

+ 18 C\* (with mutation)

1) ladder

2) pK1 / RI + PacI

3) - 9) - clones / RI + PacI

clones 1, 4-8  $\Rightarrow$  recombinant $\Rightarrow$  clone 2 - wt. $\Rightarrow$  name recombinant  
plasmid (K2).

October 27, 96

Analysis of unimpaired  
restriction~~restriction~~

1) pL2 / Sac II

2) - 8) clones

1-7 / Sac II

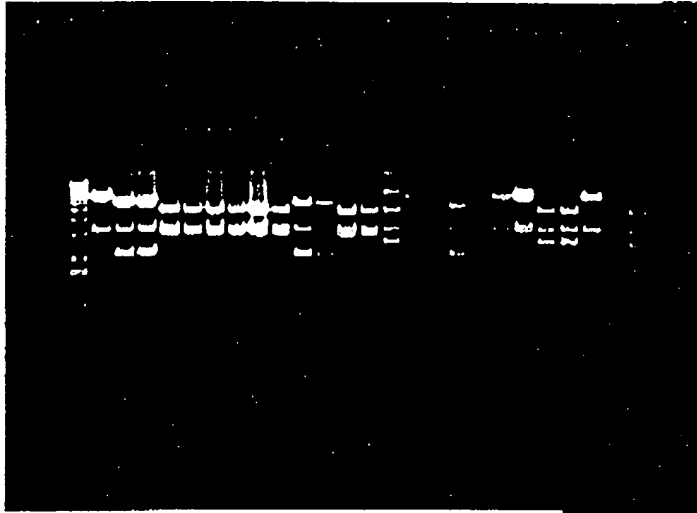
9) - ladder.

 $\Rightarrow$  1, 4, 7 - recombinant  
orientation?Cloning KDI ADP  
10/11/96

EXHIBIT

D9

October 27, 96.

22-141 50 SHEETS  
22-142 100 SHEETS  
22-144 200 SHEETS

qual. in. of min. prep  
by restriction

1) ladder  
2) p21 / KpnI

3) - (14) clones  
p21 / Bst + CMV prom.  
Bst + Bam + Klenow →

→ clones 125 - 131. Kpn

clones 25, 26, 33, 34  
the orientation that I had  
(allowing transcription)

clones 27, 28, 29, 30, 31, 32  
35, 36 → other orient.

+ clone 23 from  
other cloning site.

15 - 26) clones p21 / Bst + PCR product -  
- promoter SP-B (500 bp) with Bst I sites  
on the ends

⇒ clones 225 - 231. / KpnI

⇒ clones 27, 28 ⇒ correct orientation

+ clone 23 from previous cloning

clones 25, 31, 32 ⇒ other orientation.

Cloned SPB prom.  
in E4 10/26/96

EXHIBIT

D10

$C_{\text{pin}} \approx 70 \text{ pmoles}/\mu\text{l}$

351/1380 SmB /  $\Rightarrow$  to sequence 3ex (19 kb \*)  
 1741 Kpt use Kpt and K3  
 2004/2441 Bst

Wednesday, October 30, 96

1) Box Cloner 1, 4(?) - red preparation for sequencing

✓ 2) Purify ADP-Xba insert, purify pL1/Xba/CTP vector

|          |                                |                                    |
|----------|--------------------------------|------------------------------------|
| Ligation | insert (ADP-Xba)               | vector                             |
|          | 0.15 $\mu\text{g}/\mu\text{l}$ | 0.107 $\mu\text{g}/\mu\text{l}$    |
| control  | 15 $\mu\text{g}/\mu\text{l}$   | 5 $\mu\text{g}/\mu\text{l}$ vector |

|               |                                     |                                    |
|---------------|-------------------------------------|------------------------------------|
| 4. Experiment | 15 $\mu\text{g}/\mu\text{l}$ insert | 5 $\mu\text{g}/\mu\text{l}$ vector |
|---------------|-------------------------------------|------------------------------------|

+ 5  $\mu\text{l}$  buffer (5X) + 1  $\mu\text{l}$  ligase  $\rightarrow$  1 hr room temp.

Thursday October 31, 96

1) 253 cells tomorrow will be ready to pass  $\Rightarrow$  prepare HEBES and  $\text{CaCl}_2$  What about 2x media?

2) Sequence !!

3) Prepare red ADP-Xba insert / Ligate, transform!

4) Order primers for sequencing p2 inserts.

3) first, repeat PCR with  $\text{p2}$   $\text{p2}$ .

5) Ask Mahan about TP + drug AdS.

$$C = 7.5 \times 10^4 / \mu\text{l} = 0.075 \times 10^6 / \mu\text{l}$$

Cloning K03 ADP

EXHIBIT

D11

Sequencing

pK2 (8p19k\*)  $\rightarrow$  clone 1

- MIX — 8  $\mu$ l
- Template — 1  $\mu$ l
- Primer — 5  $\mu$ l  
(KD1 or KD3)
- $H_2O$  — 6  $\mu$ l

P.T. Temp 96°C

96°C — 30"

50°C — 15"

60°C — (4 min!)

25 cycles  $\rightarrow$ 

KD1, KD2, KD3

HEBS 2X for 200 ml

- ▼ 2 g HEPES
- ▼ 3.2 g NaCl
- ▼ 0.148 g KCl
- ▼ 0.039 g  $Na_2HPO_4$  (anhydrous)
- 0.4 g glucose

final pH = 7.0.

 $CaCl_2$  2.5M

MW = 147.00

for 100 ml

36.75 g ▼


 22-141 50 SHEETS  
 22-142 100 SHEETS  
 22-144 200 SHEETS

EXHIBIT

D12

Friday 1, November, 1986

- 1) Prepare HEBs,  $\text{CaCl}_2$ , test incubate, filter
- 2) Run  $\frac{1}{2}$  of 5 pfu,  $\frac{1}{2}$  cut with  $\text{XbaI}$ , run electrophoresis, purify fragment, ligate, transform this
- 3) Cut TP-DNA with  $\text{EcoRI}$ , check on gel.  
no fragment? repeat, take pF640 as template!!!  
take other pfu polymerase!!

Saturday 2, November, 1986

- 1) Set up cells in 6 cm dishes @ dishes.
- 2) Prepare HEP (0.1 M), NaOH (0.1 M) for cell culture.
- 3) Cut TP-DNA with  $\text{EcoRI}$ , run electrophoresis.
- 4) PCR AD-X62 with pfu, pF640 as template.
- 5) Filter sterilize Carlet's carrier DNA (salmon sperm DNA).

PCR with pfu

100  $\mu\text{l}$  template (1  $\rightarrow$  1000  $\text{E. coli}$ )  
 2  $\mu\text{l}$  primer (4)  
 2  $\mu\text{l}$  primer (5)  
 100  $\mu\text{l}$   $\text{H}_2\text{O}$   
 2  $\mu\text{l}$  bf for pfu  
 2  $\mu\text{l}$  pfu  
 4  $\mu\text{l}$  dNTP  
 200  $\mu\text{l}$

Sunday 3, November, 1986.

gus PCR product, buffer pfu?

- 1) Cut gag PCR product AD-X62, with  $\text{XbaI}$ .
- 2) Transfection pF6 100. — 20  $\mu\text{l}$  (100).  
 each TP-DNA spl + carrier — 10  $\mu\text{l}$  (100)  
 on 2 dishes (TP-DNA spl + pF2 20  $\mu\text{l}$  (200) at 2x60;

EXHIBIT

D13



Monday, 4, November 1996 (laundry small volume!)

- ✓ 1) Purify fragment ADP-dbs, Egate, transfer OHSd.
- ✓ 2) When transferring OHSd, seed cells on SOB-agar for preparing competent cells.
- ✓ 3) Order primers for sequencing plasmid.

Ligation  $\frac{\text{vect (L1)}}{\text{inset (ADP-dbs)}} = 0.1/1$   
 $\frac{\text{vect (L1)}}{\text{inset (ADP-dbs)}} = 0.01/5$

Ligation (promega)

control 1.5  $\mu$ l vect + 8  $\mu$ l H<sub>2</sub>O + 1  $\mu$ l ligase  $\rightarrow$  2h room temp.  
 exp. 1.5  $\mu$ l vect + 8  $\mu$ l fragment + 1  $\mu$ l ligase  $\rightarrow$

! Sequencing failed  $\Rightarrow$  too low ptx!!!  
 Repeat with both primers 3x more template  
 3x more primers

mix  $\rightarrow$  8  $\mu$ l  
 template  $\rightarrow$  8  $\mu$ l  
 primers 13  $\mu$ l dilute 1/10  
 take ① - 2  $\mu$ l (3)  
 ② - 4  $\mu$ l (5)  
 + 6  $\mu$ l H<sub>2</sub>O (1)  
 + 4  $\mu$ l H<sub>2</sub>O (3)

! There are no E3 polyA sites in L2!  
 May be it can use fiber's gene pA?  
 Moreover, there are no acceptor splicing site  
 in your construct, how can it be expressed?

Check literature on expression of inserts in the  
 deletion of E3.

Splicing in Ad, esp. E3 splicing.

22-161 50 SHEETS  
 22-162 100 SHEETS  
 22-164 200 SHEETS

EXHIBIT

D14

Tuesday 5, November 1996.

- 1) Repeat sequencing: 3ex + clone primers 1 and 2
- 2) Seed clones new 4ex (5 clones) on sectors.
- 3) Prepare competent cells DH5 $\alpha$
- 4) Check ~~clon~~ number insert into p21 with other restriction sites, start clone it into pBAC1.

Sequencing  $\rightarrow$  with primer 3 have a precipitate.  
with primer 1 precipitate is much less

Wednesday 6, November 1996

- 1) Extract plasmids from clones 4ex (5 clones)  
check if they are restriction sites
- 2) 4) - from yesterday.

Thursday 7, November 1996.

- 1) Seed clones for liquid culture for minipreps with Wizard Plus miniprep kit for sequencing
- 2) PCR with pFur of pBAC1 (primers 4, 5)

25  $\mu$ l - H<sub>2</sub>O  
 1  $\mu$ l - template pBAC1 (no)  
 10  $\mu$ l - buf. pFur  
 1  $\mu$ l - primer 1  
 1  $\mu$ l - primer 2  
 2  $\mu$ l - dNTP  
 1  $\mu$ l - pFur  
 10  $\mu$ l - DH5 $\alpha$

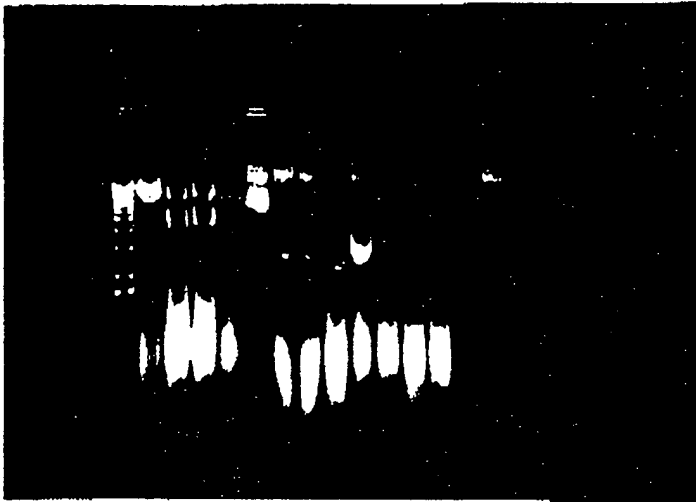
- 3) Prepare vectors Lit/Xba and pBAC1 / ~~SPB~~ - large fragment
- 4) Check env in p21 (Nde I)
- 5) Check env wanted and for MW?
- 6) PCR of inserts into p21  $\rightarrow$  with SPB it is possible to check orientation!

EXHIBIT

D15

Run 11, 5 lanes gel electrophoresis.

- 1) - Ladder
- 2) - 21
- 3) - 123 (buffer D, orange)
- 4) - 125
- 5) - L1
- 6) - 10) 1-5 clones rep.
- 11) - 14) L1/864 prep.
- 15) - pBAG11 / RI prep.



Conclusions!

1) it's on with caw  
promoter L1/864

2) it's on with  
pBAG11 RI

3) L1/864 clones → trash  
4) L1/864 → trash.

November 10, 1996, Sunday, November 11, 1996, Monday

- 1) Split 293 cells on new flask.
- 2) Overlay 293 dishes with neutral red.
- 3) Run electrophoresis of Wizard minipreps and L1/864.
- 4) PCR of clones + electrophoresis.
- 5) ~~PCR~~ CIP pBAG11/RI, cut from the gel?
- 6) Cut p21/cmv with EcoRI, get the fragment from the gel?
- 7) Change the medium in 293 flask.
- 8) (New prep. of L1) Clean up old preps?
- 9) Seed 54, 57 and L1 for Wizard miniprep!

EXHIBIT

D/6



22-141 50 SHEETS  
22-142 100 SHEETS  
22-144 200 SHEETS

Tuesday 12, 1996

Cannot cut DNA after Wizard kit miniprep!

- 1) 123 - with EcoRI - put, take from gel  
 2) L1 - with XbaI - large fragment!

Wednesday 13, 1996.

- 1) CIP pBAG11, cut fragment from gel, cut fragment 123/R.  
 2) Try to cut L1 with XbaI, if not successful → seed L1 pN

- 3) Ligate from 1).

12 μl vec + 5 μl insert + 1 μl exon  
 + 1 μl H<sub>2</sub>O - control

L1/XbaI not in single band. 2) 10 μl digest with XbaI  
 → extract with W. spool → seed it in ligase culture

pBAG11/R1/CIP - A - fragment - not single band →  
 be ready to distinguish between all this  
 forms.

Thursday  
Tuesday 14, 1996.

- 1) Transform DMSO cells ligase mix from yesterday.  
 (Try to obtain high efficiency!!!)

- 2) Extract L1 with Wizard, cut XbaI, run together  
 with yesterday's L1/XbaI (one) on the gel.

- Try PCR! Maybe you can use it for screening ???

- Sequencing with new batches of 32!!!

- PCR (Taq polymerase)

for 150 μl

15 μl buffer  
 3 μl dNTP  
 4 μl MgCl<sub>2</sub>  
 1 μl Taq pol  
 130 μl H<sub>2</sub>O

- 1) 123  
 2) 125 } primers 10, 11.

- 3) 223  
 4) 227 } (insert in p21) primers 8, 10

- 5) p21 - control (-)

- 6) 223  
 7) 227 } primers 9, 11  
 → check orientation

- 19 54 } primers 6, 7.

- 11) 52

- 12) pL2 - control (-)

(13, 14, 15) cut SmaI with PstI  
 L9 - control

19 reactions

15 (10-11)  
 2 (12-13)  
 4 (14-15)

225 with SmaI 10/11/12/13  
 227 with SmaI 10/11/12/13

EXHIBIT

D17



1402 South Grand Blvd.

St. Louis, MO 63104

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FAX: 314-773-3403

**SAINT LOUIS  
UNIVERSITY**

Department of Molecular  
Microbiology & Immunology

Health Sciences Center  
School of Medicine

**Facsimile Transmittal**

Date: 12-19-02

From: William Wald

Phone: 314-577-8432

Fax: 314-773-3403

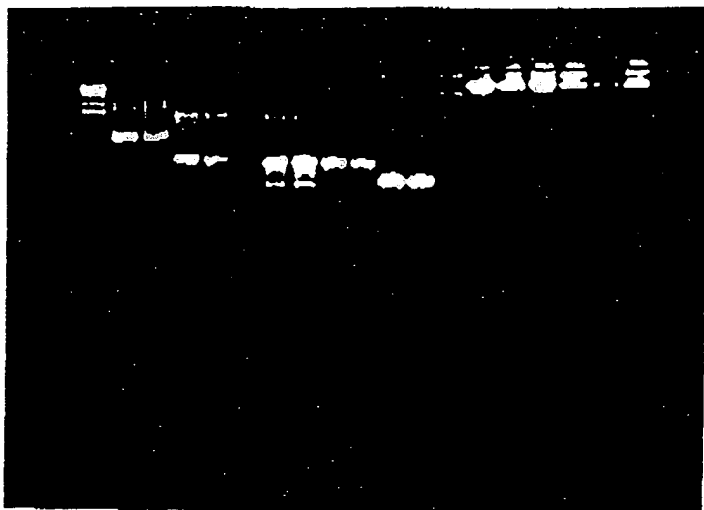
Deliver to: Dan Kasten

Phone: Fax: 552-7305

Total number of pages including this page: 15

Message:

see explanation  
of the picture  
on  
previous  
page.



Conclusions: everything is fine  
except that Antisense didn't cut DNA  
(because of buffer 4, I think).

Friday 15, 1996.

- 1) ~~Transform~~ L1! Clean up L1 preps., try to cut with Xba
- 2) Seed lanes of pR1611 + 123 (Cuv).



22-141 50 SHEETS  
22-142 100 SHEETS  
22-144 200 SHEETS

EXHIBIT

D18

MAE 299. W138

Friday (1:00 /

Monday, 18, 1996.

✓ 1) Analyse clones (1-24) pBUG11/RI + CMV (128 clones)  
run on gel and then cut with NdeI

✓ 2) Phone about SrfI.

✓ 3) Transform L1.

✓ 4) Sequence PCR

✓ 5) Prepare TP-DNA, TP-DNA/RI, Ad5 DNA, Ad5 DNA/RI

✓ 6) Split 293 to dishes for transfection.

Sequencing.

✓ 8 µl pre mix

✓ 5 µl p31 (0.5x)

✓ 1 µl primer (100 µl)

✓ 6 µl H<sub>2</sub>O

✓ 20 µl oil

circling.

✗ pBUG11 gave 2 small fragments instead of 1.  
what is it?

clones gave incomplete digestion after PEG precipitation → O/N Met

Tuesday, November 19, 1996.

1), 3), 5), 6) from yesterday.

if fail in 1), do PCR with 10, 11 primers!

Freezer at CF room 3 shelf from top

left drawer

upper box (-4)

tube with 918 on cap

(VS 20.) on side

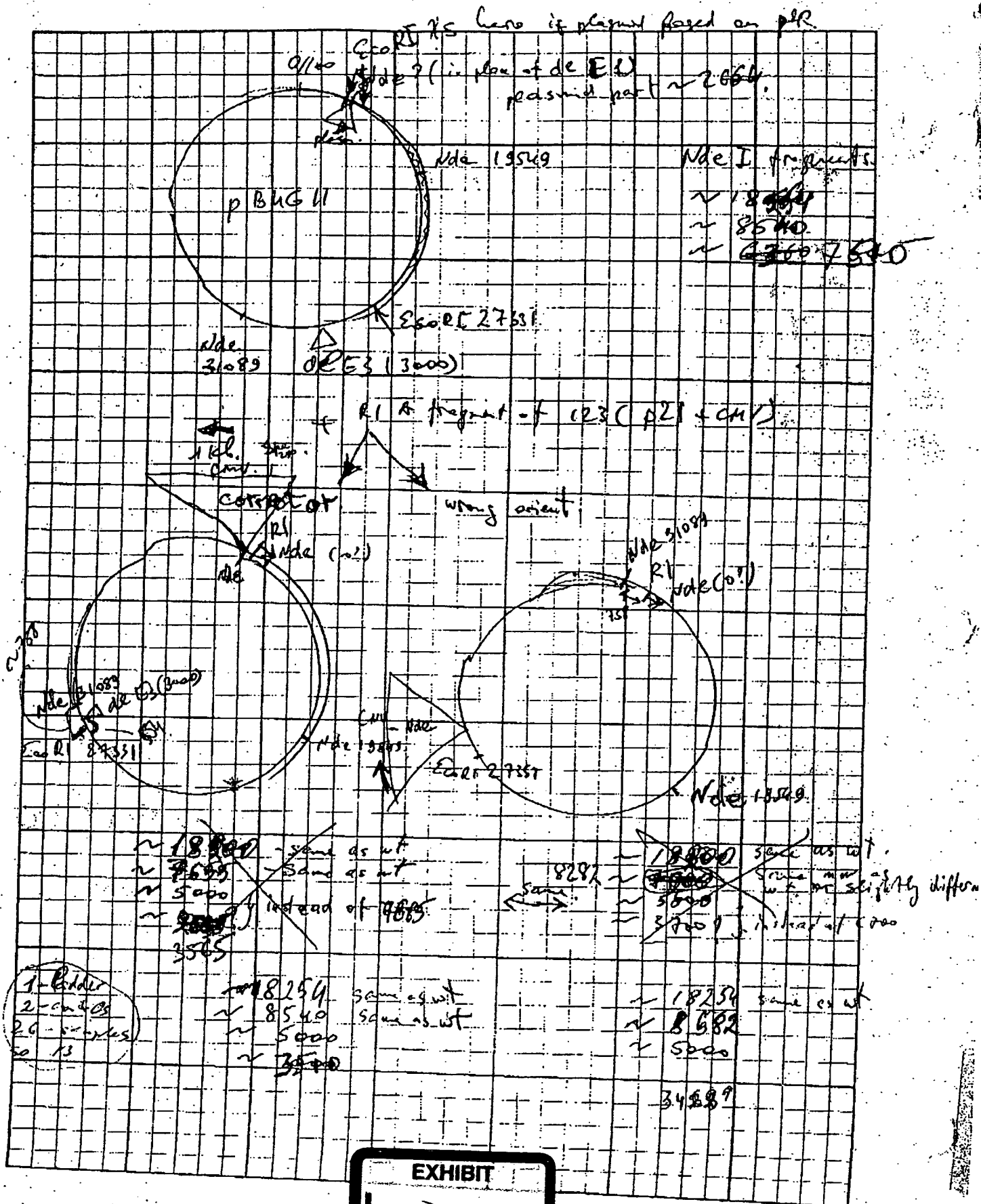
→ Met  
CSA

EXHIBIT

D19

50 SHEETS  
100 SHEETS  
200 SHEETS







← But it is not like this with EcoRI and NdeI  
 because you were able to see  
 ↳ It's like this leave NdeI digestion of p21  
 gave linear form.

⇒ Incomplete digestion? (with NdeI)

⇒ cut with EcoRI  
 and PacI + BamHI (pBHG 11 as control).

1) ladder 2) pBHG 11 / NdeI  
 minipreps: (6 ex)

3-26) - clones 1-24

⇒ clones 4, 7, 9, 14, 18, 21  
 → trash.

clones 1, 2 and other  
 with same pattern are  
 with correct orientation of  
 insert?!

Partial digestion with  
 NdeI!

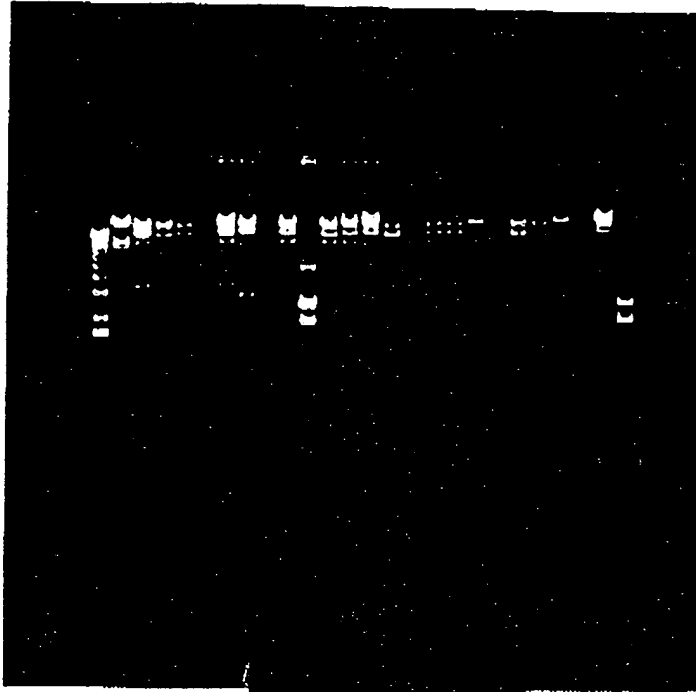
Bottom picture is the  
 same.

or cloning in partially  
 digested pBHG 11  
 or star activity digested?

take to analyze RI

1, 3, 5, 6, 17, 19, 21

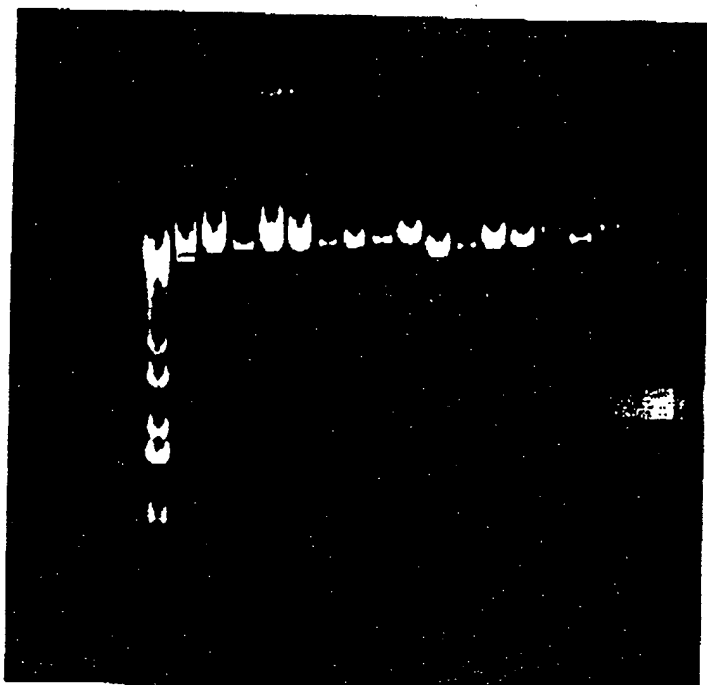
50 SHEETS  
 100 SHEETS  
 200 SHEETS



EXHIBIT

D21

Pac + Bam → NEB Buffer (4) - green. both enzymes  
~~NEB Buffer (4) - green~~  
 Eco RI → (multi core) → Buffer 4, Promer.



1) Buffer  
 2) BAC / R1  
 3) 1  
 4) 3  
 5) 5  
 6) 6  
 7) 17  
 8) 18  
 9) 21

(10) BAC / Bam + Pac  
 (11) 1  
 (12) 3  
 (13) 5  
 (14) 6  
 (15) 17  
 (16) 19  
 (17) 21  
 18

Conclusions! 17, 21 are OK by Eco RI digestion  
 others are result of Eco RI star activity  
 when preparing vector.

Pac + Bam → pBAC II gave more fragments than  
 expected.  
 all clones are similar and different  
 from pBAC II. Wrong orientation?  
 They have different orientations.  
 Pac did not work in clones?

17, 21 are not OK inserts are too small. 11/27/99

EXHIBIT

D22

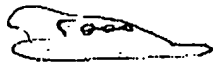
2 orientations

10000

11000

8000

7500

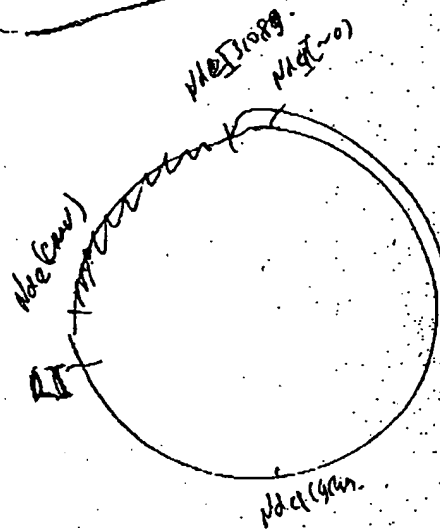
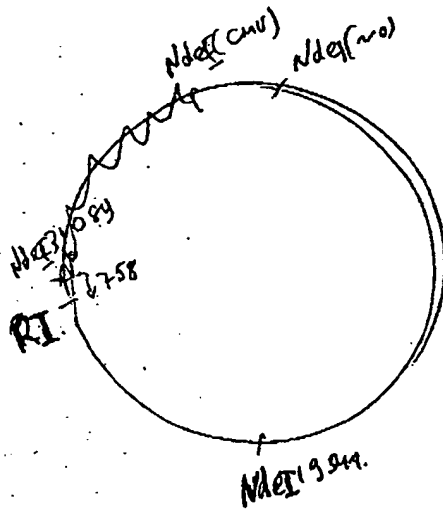
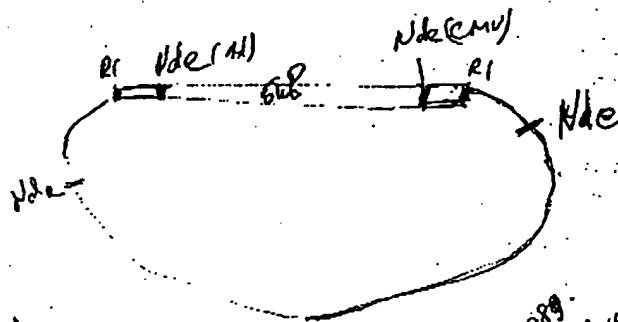


3000

2700

you can explain  
it just in the case  
of larger fragments  
are made of  
partial hydrogens

23-141 50 SHEETS  
23-142 100 SHEETS  
23-144 200 SHEETS



EXHIBIT

D23

it is mixture of 2 plasmids  
and  $\Rightarrow$  there are just 2  $\Delta$ dc sites?

EXHIBIT

D24

Wednesday, November 20, 1996. Thursday 21 November

- 1) Seed L1 for mini (unidi) prep. with Wizard!
- 2) Cut TP-DNA A45 with Eco RI, check on E/F, dilute against TE.
- 3) Amplicon A15 DNA, check amount, cut Eco RI, phenol, precipitate.
- 4) Split 293 to dishes for transfection.
- 5) Sequence clones.
- 6) Prepare new vector pBHG/RI/ZIP, ligate with 123/RI insert.
- 7) Seed 17,21 clones for further characterization. Extract with Wizard kit!
- 8) Cut pBHG with RI, Nde, (Bam, Pst), (Bam + Kc?), (Kc + Pst?)
- 9) Check SfiI site in pEG140, pBHG, p2, something?

Friday, November 22, 1996.

- 1) Transfection of 293 with pL2 + TP-DNA A45/RI or ~~WIZARD~~
- 2) Extract 17,21 and L1 with Wizard mini-prep.
- 3) Sequence clones.

seq #2 3ex, clone 1 (31) - primer 10, 2.

seq #3,4 2ex, clone 23 (223) - primers K10, K11

seq #5,6 1ex, clone 23 (123) - primers K10, K11

seq #7,8 5ex, clone 4 (54) - primers K6, K7.

8ul premix  
5ul template (0.58)  
1ul primer  
6ul H<sub>2</sub>O

40ul oil  
circling

EXHIBIT

D25

Monday, November 25, 1996.

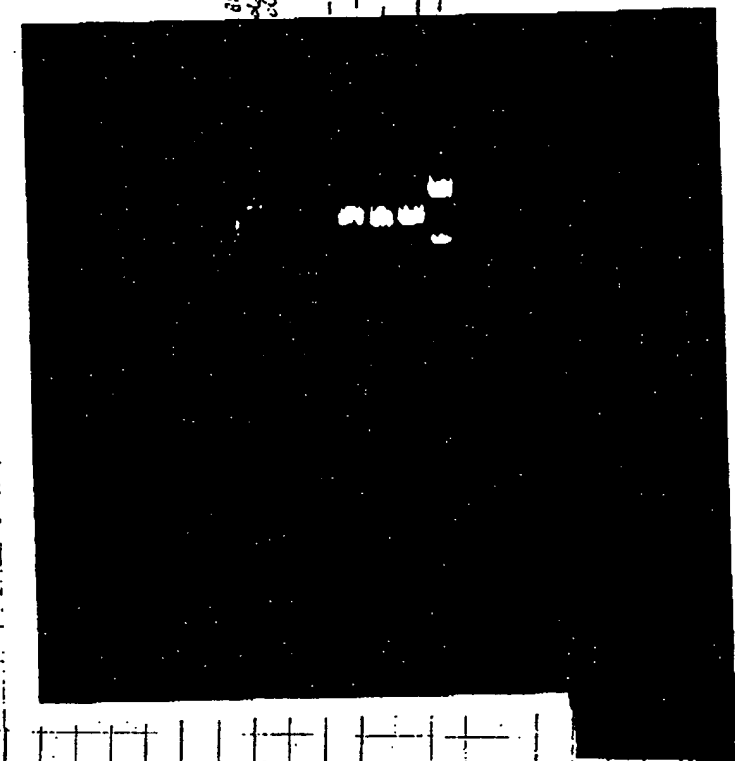
- 1) Cut new preparation of L1 with Xba, check on E/F.
- 2) DIP / BAC11 / R1
- 3) Check amount of L15 / R1
- 4) Cut 17,21 with ~~Bam + Srf~~ <sup>Bam + Srf</sup>, pBAC11 - control → no fragment 5858
- 5) Check plasmids with Srf I.

Srf I - site → 250 in CMV promoter.

⇒ in right orientation → fragment ~ 5800 bp  
~~about 5800 bp~~⇒ in wrong orientation fragment ~ 1000 bp.  
wt - linear form.

Bam + Srf  
BAC11 / R1  
L1 / Xba  
BAC11 / R1  
L15 / R1

- 1) ladder
  - 2) BAC11 ? Bam + Srf
  - 3) clone B
  - 4) clone C
  - 5) L1 - D
  - 6) L1 / Xba - C
  - 7) L1 - M
  - 8) BAC11 / R1 (buffer H)
  - 9) L15 / R1 (309?)
- Protease buffers



Bam + Srf hydrolysis (buffer H) - stronger picture, same as before Bam + Pac → M.B.  
D Bam sites or star activity?

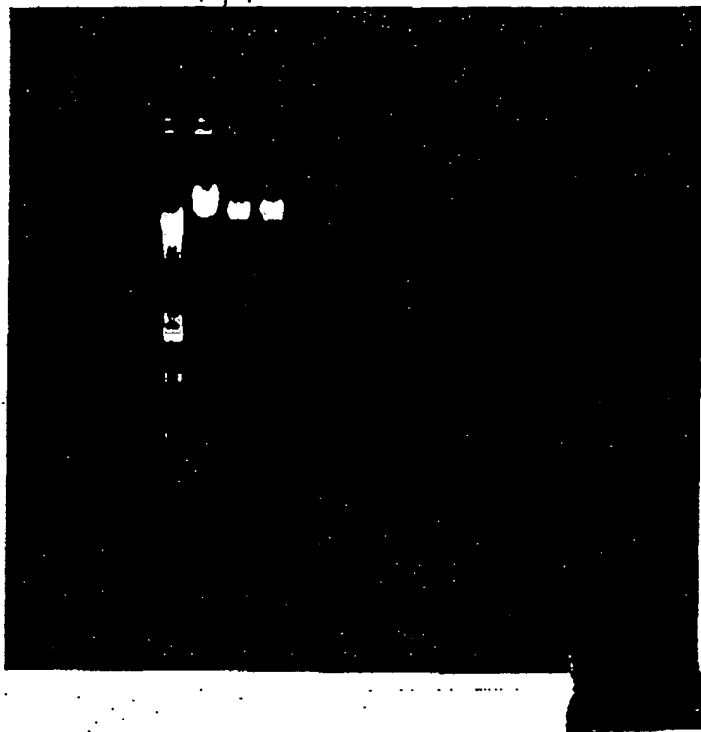
EXHIBIT

D26

22-141 50 SHEETS  
22-142 100 SHEETS  
22-144 200 SHEETS



- 046119  
- done 17  
- done 21



1) ladder

2) 04611A

3) done 17 Spe I

4) done 21

there are no fragments  
~ 6000 bp →  
→ wrong orientation  
or what?  
→ repeat cloning.

EXHIBIT

D27

Thursday, December 27, 1996

- 1) pGEM1/CIIP → extract from gel  
 L1/Xba → CIIP → extract from gel  
 ligate to inserts o/n
- 2) pGEM1/CIIP (new prep, 0.1/1) → 12 μl  
 1.2.3 insert (old prep, 0.05/5) → 7 μl  
 2 μl buffer  
 1 μl enzyme  
 o/n 16°C
- 3) pL1/Xba CIIP (new prep, 0.1/5) → 12 μl  
 3(PCR)/Xba insert (new prep 0.1/5) → 5 μl  
 → 2 μl buffer  
 → 1 μl enzyme  
 o/n 16°C

Wednesday, 28, 1996

- 1) Transform DH5α cells with ligation mixtures from yesterday, try to obtain high efficiency!  
 plate cells in duplicates, 1/10, and all
- 2) Plan scheme of experiments to obtain Berger plasmids, check everything, start cloning.
- 3) Split 293 in flask

Friday 30, 1996

- 1) Seed clones 6ex, 7ex in liquid culture and agar plates
- 2) Split 293 cells, overlay 293 dishes with transfection

EXHIBIT

D28



Saturday, November 30, 1996.

1) Extract Gex 25-36 with Wizard kit, cont. with  
SpeI Extract wex 1-29 with  
cont. with Sac II.

2) from yesterday.

SpeI - NEB Buffer II

Sac II - buffer C/Orange

3) Check preferences about splicing in Aa, especially  
in ES and fiber!

4) Start with genes IL and IV?

\* Filter NEBS and CaCl<sub>2</sub> from freezer 2 times!

(WB)

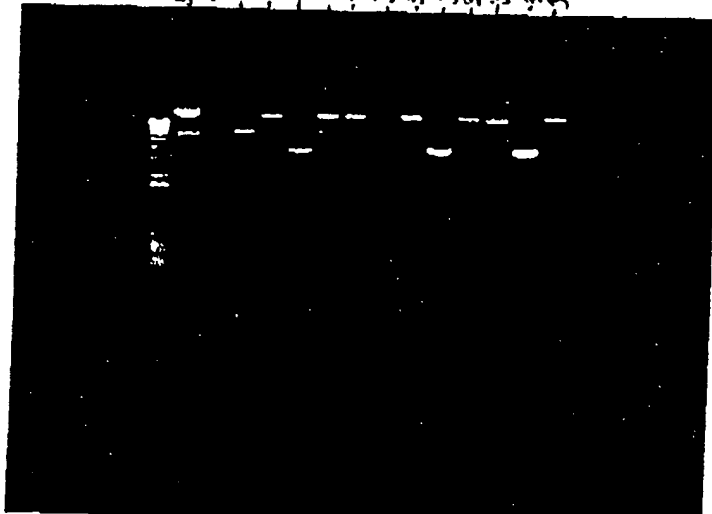
There is no SpeI site in CMV promoter according  
to my sequence  $\Rightarrow$  may be clones 12 and 21  
from previous cloning are OK?

Sunday, December 2, 1996.

1) Check new Gex clones with EcoRI, select recombinants

2) Check new Gex clones with XbaI, select recombinants

BglII/A  
 BglII/A  
 25  
 26  
 27  
 28  
 29  
 30  
 31  
 32  
 33  
 34  
 35  
 36



1)

1) Ladder

2) BglII/R1

3) R2/R1  $\rightarrow$  A fr.

4)  $\rightarrow$  25-36

Recombinants:

26, 28, 29, 3, 33, 36

2) Cannot see  
fragment  
on XbaI hydrolysis

EMIB

1234

3) Hex  $\text{SacII}$  (1 site in ADP), no sites in CF  
 + RI (0 sites in ADP), 1 site in L1  
 expected fragments correct orientation ~1261  
 wrong orientation ~1561  
 orange buffer (C)

4) Gen clones Pcc + Bam.  
 1 h Pcc (buffer NEB1 + BSA)  
 ↓ heat inactivate  
 ↓ + 1X buffer (D) + Bam → 1 h → E/E  
 (A)?  
 (D)?

NEB 1 - 10 mM Trispropyl-HCl, 10 mM  $\text{MgCl}_2$ , 1 mM DTT (pH 7.2)

BamHI - 150 mM NaCl, 10 mM Tris HCl, 10 mM  $\text{MgCl}_2$ , 1 mM DTT (pH 7.9)  
 unique NEB  
 buffer.

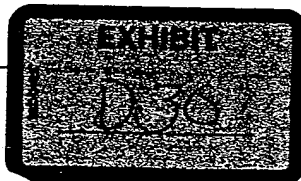
buffer D - pH 7.9, Tris HCl - 6 mM,  $\text{MgCl}_2$  - 6 mM, NaCl - 150 mM  
 orange pH 7.9  
 D - yellow.

Buffer C (orange) pH 7.4, 10 mM Tris HCl, 10 mM  $\text{MgCl}_2$ , 50 mM NaCl

MCORE (white) pH 7.8, Tris HCl - 25 mM  
 acetate

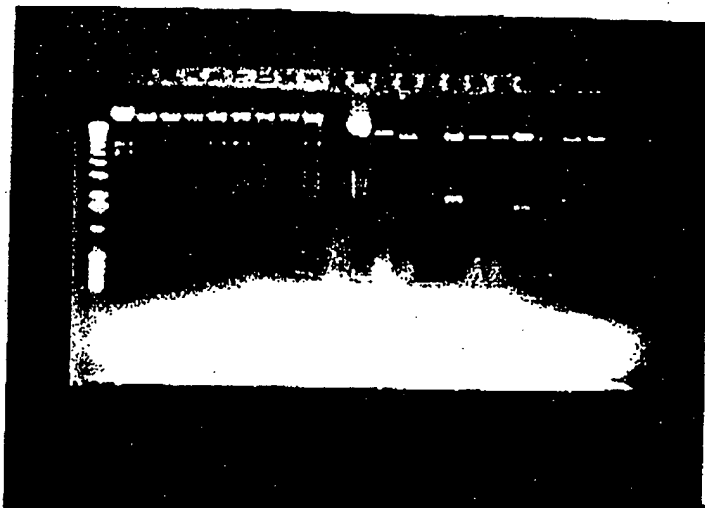
100 mM K acetate, 10 mM Mg acetate

Take orange 2X?



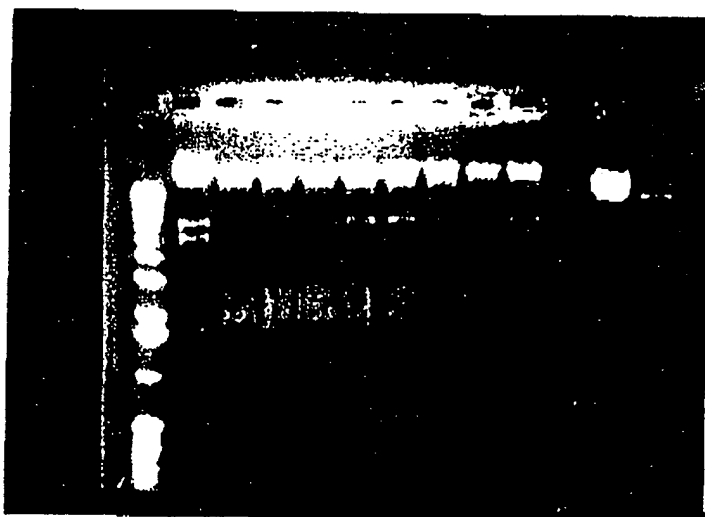
Cloned UDS AOP

12/2/96

22-141 50 SHEETS  
22-142 100 SHEETS  
22-144 200 SHEETS

- 1) ladder
  - 2) pUC11 / Pac + Bam
  - 3) 17
  - 4) 21
  - 5) 26
  - 6) 28
  - 7) 29
  - 8) 31
  - 9) 33
  - 10) 36
  - 11) blank
  - 12) L1 / Sac II + R1
  - 13) - 241 - clones
- hex (1-12) / Sac II + R

the same gel,  
higher magnitude.



Conclusions:

hex -

- clones

2, 5, 6, 7, 8, 10 -

- correct orientation

clones 4, 9, 12 -

- wrong orientation

hex - absolutely  
unclear  
what is happening.

yellow ←

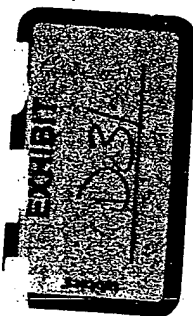
Tomorrow try to cut with Nde I,  
take 10 µl of plasmid, good activity  
of Nde I.

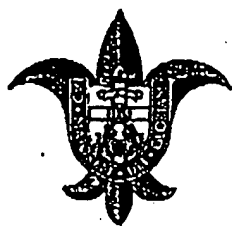
Try some other enzymes? (Not Pac  
not Bam)

universal  
IX.

←

for example Spe I, Srf I.  
correct or → 448





1402 South Grand Blvd.

St. Louis, MO 63104

Phone: 314-577-8432

FAX: 314-773-3403

**SAINT LOUIS  
UNIVERSITY**

Department of Molecular  
Microbiology & Immunology

Health Sciences Center  
School of Medicine

**Facsimile Transmittal**

Date: 12-19-02

From: William Wald

Phone: 314-577-8432

Fax: 314-773-3403

Deliver to: Dan Kaster

Phone: \_\_\_\_\_

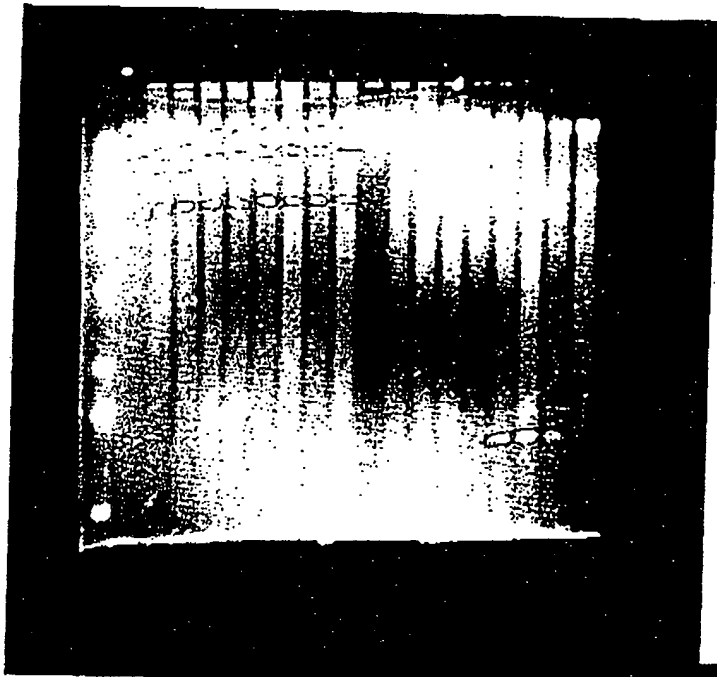
Fax: 552-7305

Total number of pages including this page: 18

Message:

Tuesday, November 5, 1996

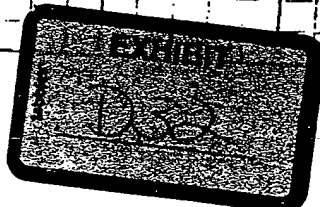
1) cut 600 clones with Kdc (Buf. I, yellow)  
and Grf + Spe I (Hoffman universal TX)



Conclusions: 17, 21, 24, 37, 33 - one orientation  
26, 28, 36 - other orientation

If consider the previous picture, 26, 28, 36 → correct orientation  
because there was additional 6 kbp from Bam  
fragment.

Obviously, there is Spe I site in CMV  
didn't give fragments because they are  
wrong orientation (on Spe I hydrolysis) 17, 21



Wednesday, 4, 1986

1) Cut cex clones with SpeI. (bigger Multicore)

22-141 50 SHEETS  
22-142 100 SHEETS  
22-144 200 SHEETS



1) ladder

2) pB4611 / ~~Spet~~ Spet

3 - 100

17, 21, 26, 28, 29, 31, 33, 36  
- SpeI

there is fragment

6 kb in (26, 28, 36)

⇒ correct orientation

there is fragment

1200 bp in 29, 31, 33

⇒ wrong orientation

17, 21 - junk! Look  
also to upper fragment  
on the previous picture.

Conclusion: When everything is OK, it's OK.

Friday, December 6, 1986.

1) Cut pFG140 → BamHI + NdeI fragment (9437)  
(contains EcoRI site, other fragments do not).  
other fragments } ~ 2500 - if there is no NdeI in pFG140  
at 1300 }  
6000 } 2043  
if there is NdeI site in pFG140.

at 223 (p11508) with NdeI + BamHI - vector

2) Spet 293 cells.

3) ~~Reseed~~ Reseed clones to new dish.

Monday December 9, 1996.

- 1) And 3) 4) from Friday.
- 2) Prepare LB for preparative growing of *hex*,  
Inoculate LB overnight (15 min).
- 3) Prepare the *hex* pH (8.5) (8.0) (7.5) (7.2),  
autoclave.

Can use 1109/1107 for confirmation to attempt  
to obtain a v. ns with *E. coli* promoter  $\rightarrow$  SP-B promoter  
substitution.

- 4) Start cloning *EcoRI*-*Sal* fragment pFG140.  
into pK2 (gp19C+)

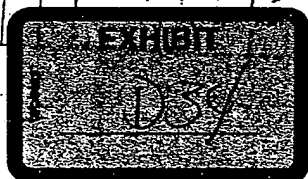
pFG140  $\xrightarrow{\text{BamHI} - \text{Nde} - \text{Buffer}}$   
 $\xrightarrow{\text{Sal} - \text{EcoRI} - \text{Buffer}}$   
 923 (p1 + SP-B promoter) - *Nde* - *Bam* HI - Buffer - vector  
 pK2 (31, gp19C+) - *EcoRI* - *Sal* I - Buffer - vector.

pFG140 *Sal* - *EcoRI* - expected fragments:

17869  
 there is  $\rightarrow$  (10585)  $\leftarrow$  that is what I need,  
 no *Sal* contains *Bam* HI site!  
 in pK2, 6905  
 if 379  
 there is *Sal*, then instead of 17869 I will have  $\sim$  9000.  
 $\sim$  8869

Wednesday, December 12, 1996.

- 1) Extract manip prep of *hex* (clone 26)
- 2) Extract Wisard *hex* (clones 2, 5)
- 3) Prepare vectors and fragments from yesterday,  
+ *hex* (2) *Sal* + *RI*.
- 4) Check *hex*, *hex* with *XbaI*; *RI* + *Sal* II (sequence??)



223/ Bam-Hle → 1 min 3000000 79  
 Bam-Hle start def. 100% M.S. there is 2 site  
 for Bam-Hle? It looks like  
 there is 2 site  
 It also can explain  
 fragment on Bam-Hle  
 P1016 (1) and degrade by  
 liso.

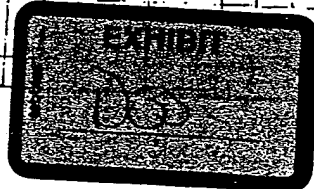
→ 5) ⇒ Seed 42, 45 and 223  
 WMP

13) Transformation (7 ex. int)  
 P1016 for EORI → 8\* (10Kb) (0.1/10)  
 P1016 (10Kb) (0.1/10) → Atr (10Kb) (0.1/10)

14) 10 µl vect + 10 µl 1000  
 7e. 10 µl vect + 10 µl 1000 + 2 µl AT + 1 µl liso

Thursday, December 12, 1996

- 1) Take 626 plasmid from CoCl<sub>2</sub>-E1Br → 2nd spin
- 2) Extract 223 WMP (42, 45 - didn't grow)
- 3) Transform <sup>1000</sup> E. coli mix from yesterday, 6)
- 4) Split 223 cells
- 5) Transform 42, 45 make WMP prep immediately!!  
 (Seed 42, 45) again → 1000 disp this time!  
 Need to 1) Sequence 42, 45 = need WMP  
 2) Grow up 54 (42 + ADPI) → ready for cotransfection!





Friday, December 12+1, 1996.

1) Purify 626 from cell.

2) Check amount of  $\lambda$ -PI fragments  $\lambda$ -PI fragments  $\lambda$ -PI fragments

Saturday, December 14, 1996.

1) Prepare LB, sterile  $\lambda$ -phage, sterile TE

2) Dissolve 626 prep in sterile TE. Check plasmid with

3) Split 293 cells to dishes. Transformation 626+ left arm

4) Repeat transformation of old prep. of  $\lambda$ -phage (left arm)

5) Transform DMSO (new prep) with 42, 45

7ex, 7c  $\Rightarrow$  10  $\mu$ l of each to 100  $\mu$ l of old DMSO cells

42, 45  $\Rightarrow$  0.001 g of each to 100  $\mu$ l of new DMSO cells

Monday, December 16, 1996.

7ex, 7c  $\Rightarrow$  no clones again, efficiency of transform is

very high.  $10^4 \times 10^3 \approx 10^7$  / 1/2 pl  $\Rightarrow \sim 10^8$  / pl

What's wrong? Ligation? Not optimal ratio vector/insert (?)

pBSK was pBSKS (?)

1) Dissolve 626 in sterile TE, check on EIE amount and quality of the plasmid, check other plasmid as well (left arm)

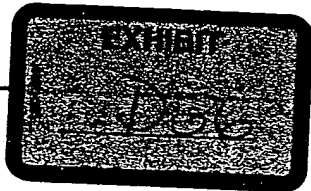
2) Transformation of 293 with 626+ left arm.

3) Ligate 7ex again, transform DMSO, 5  $\mu$ l vector transform CMV/TTF1. 15  $\mu$ l insert

4) Seed 42, 45 to liquid culture (LB).



22-141 50 SHEETS  
22-142 100 SHEETS  
22-144 200 SHEETS



60r

PKRV - left arm plasmid (4.5  $\mu$ l) 7  $\mu$ l (10 $\times$ )  
 pBB2 - (PONGII ENZYME) (0.58  $\mu$ l) 100  $\mu$ l (50 $\times$ )

✓ 1.5 ml 2X HES

✓ 1200  $\mu$ l H<sub>2</sub>O

✓ DNA (107  $\mu$ l)

✓ 150  $\mu$ l CaCl<sub>2</sub>

✓ 3 ml  $\rightarrow$  for 6 drops

2X Overlay

(for 100 ml overlay)

50 ml 2X DMEM  
 5 ml 7.5% NaHCO<sub>3</sub>

2 ml FCS

1 ml 100 $\times$  PS

4.3 ml 1.8% Noble agar.

Tuesday, December 17, 1996.

Tex  $\rightarrow$  no clones. Something in fragment preps. interfere with ligation? (It is possible, because of this red colour trial)

- 1) Seed i) CMV/ITTE, 54 (L2 + ADP) into large scale  
 ii) TK (34)  $\rightarrow$  small scale guide culture + WGA.

- 2) Extract 42, 45 with Wizard mini prep, then  
 seed (42, 45) for medium scale prep.

repeat  
 preparation  
 of this  
 fragments.

- 3) Try precipitate fragments 140/PR, 31 PR to purify them.

- 4) Try partial digestion p223 Bam Nde  
 (Nde - complete, Bam HI - partial  
 dephosphorylate, try cloning with 140 BN B
- Try to  
 clean  
 with  
 Wizard  
 clean up  
 system.

EXHIBIT

1-1237

22-141 50 SHEETS  
 22-142 100 SHEETS  
 22-144 200 SHEETS

Wednesday, December 18, 1996.

Thursday, December 19, 1996.

Cut pFG140 → (Bam + Nde) (Buffer D)

→ Sal I + Eco RI (Buffer D)

Cut 223 with Nde → then with Bam HI (partial) →  
(complete) → plant → CAP → vector. Run gel.

Cut 31 with Sal + Eco RI (Buffer D)

Cut 223 Eco RI (Buffer D)

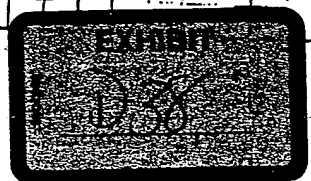
31/R1, 140/R1, 223 Nde, 223 R1, 140/Bam Nde

Sunday, December 22, 1996.

- 1) Overlay dishes 293 with neutral red.  
Split 293 cells to dishes → repeat transfection.  
Seed frozen 293 cells for last passage.
- 2) Check 3, 31/Sal, 31/R1, 31, Sal + R1 to estimate  
molecular weight of the plasmids, 223/Nde, and part of  
with Bam HI.
- 3) Cut fragments from gel, ligate.
- 4) Seed (CMV/TTA) 59 for large scale liposome culture LB.
- 5) Seed (P6) plasmid for WAP.

Sunday, December 29, 1996.

- i) Check i) CMV/TTA - with Pst I, Sac I.
- ii) P5 ? → Hind III + Xba I, Pst I.
- iii) P54 (map) - Pst I, 2% agarose.
- iv) 42 - Xba I  
45 - Xba I



- \* 2) Split 293 cells to dishes for transfection.  
 3) Cut 1101/1107 DNA with EcoRI.

Transfection:

- 2 dishes pEG1us.  
 → 2 dishes E1 + pBAG11  
 → 2 dishes E1 + pBAG11 ΔEGp CMV promoter.

First attempt  
 to make KO1  
 12/04/96 (T)

500 μl 2x HEBs + 450 μl H<sub>2</sub>O + 20 μl pEG1us.  
 + 1 + 20 μl BAG11 + 10 μl GCL  
 + 1 + 20 μl BAG11 (EGpCMV)

Precipitate is coarse  
 orange where E1 plasmid is used → purify it (SCE long)

Saturday, December 4, 1997.

- ↓ 1) Cut 1101/1107 with EcoRI.  
 ↓ 2) Split 293 to dishes, cotransfect 1101/1107 with L2  
 ↓ 3) Pick up this ugly things resulting plaques + to plate to try to grow up a plaques  
 ↓ 4) Overlay dishes with NR.

Overlay 2x 200 μl

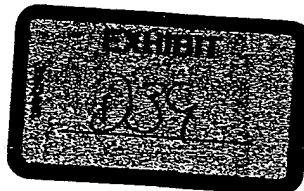
200 ml 2x DMEM  
 4 ml 2x PS  
 2 ml 5% yeast extract.  
 3 ml FBS  
 10 ml HS  
 2 ml 100x chloramphenicol  
 20 ml bicarb 7.5%

Transfection. 1101/1107 (RT) + control (H<sub>2</sub>O)  
 20x + L2 (20x)  
 + 54 (20x)

4 ml of each transfection mixture

2 ml of 2x HEBs.  
 1.8 ml of H<sub>2</sub>O  
 DNA  
 + 200 μl GCL 2.5M.

22-141 50 SHEETS  
 22-142 100 SHEETS  
 22-144 200 SHEETS



Wednesday, 8, 1996.

- 1) Spin left hand plasmid in  $\text{CaCl}_2$  gradient 2x. fine.
- 2) Split 238 to dishes for transfection.
- 3) Cut 31 with  $\text{EcoRI}$   $\rightarrow$  complete digestion and with  $\text{SalI}$   $\rightarrow$  fragment dephosphorylate.
- 4) Cut 225 (21 + SP6 prom) /  $\text{PstI}$  with  $\text{KpnI}$  / partial  $\rightarrow$  dephosph.
- 5) Fragments (no  $\text{SalI}$  +  $\text{PstI}$ ),  $\text{KpnI}$  +  $\text{PstI}$   $\rightarrow$  cut from gel.
- 6) Ligate. 7x  $\text{PstI}$  overnight.
- 7) Precipitate 1101/1102 DNA end time, cut with  $\text{EcoRI}$ !
- 8) Sequence 42.
- 9) Run experiment with TK gene.

Thursday 9, 1996

- 1) Transform Karl's left-hand plasmid
- Monday, 14, 1996

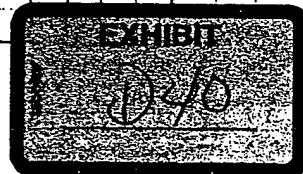
Transfection

|  |       |
|--|-------|
| 5x $\text{KpnI}$ (5 $\mu\text{l}$ ) + 15x 826 (15 $\mu\text{l}$ )      | (1:3) |
| or 10x $\text{KpnI}$ (10 $\mu\text{l}$ ) + 10x 826 (10 $\mu\text{l}$ ) | (1:1) |
| or 5x $\text{KpnI}$ (5 $\mu\text{l}$ ) + 20x 826 (20 $\mu\text{l}$ )   | (1:4) |

Ligation 8x 225 /  $\text{PstI}$  +  $\text{KpnI}$  (partial) C + P  
+ 140 /  $\text{KpnI}$   $\text{PstI}$  fragment

Thursday, 17, 1996.

- 1) Sequence 42, primers (21) 4, (22) 5  $\rightarrow$
- $\Rightarrow$  sequence  $\Rightarrow$  KD9, 4510.
- $\Rightarrow$  No sequence at all!
- What is it?



8 $\mu$ l premix  
 3 $\mu$ l p42  
 7 $\mu$ l primer (4 or 5)  
 8 $\mu$ l H<sub>2</sub>O

circ. lig

Monday, 20, 1996.

8 ex ( p223 / complete hydr. Nde + Partial Bam )  
 ( + B. fragment pFG140 / Bam + Nde. )

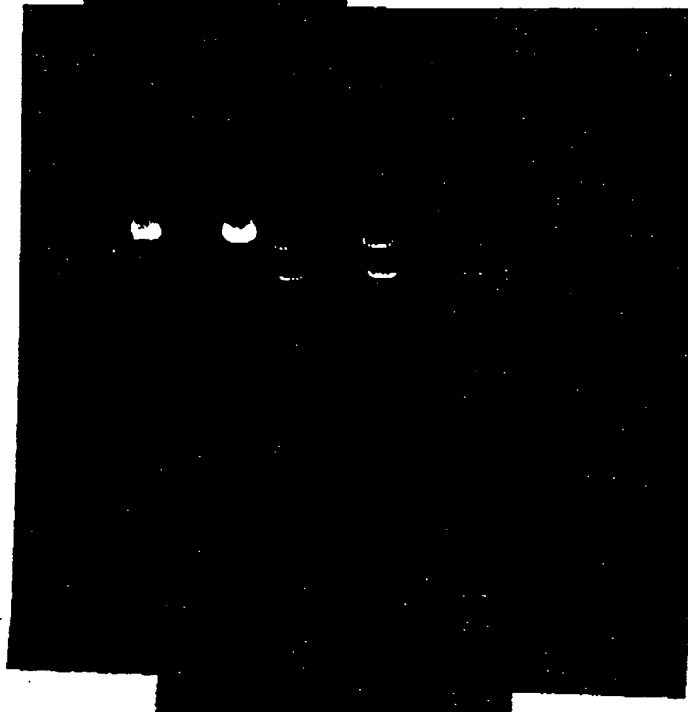
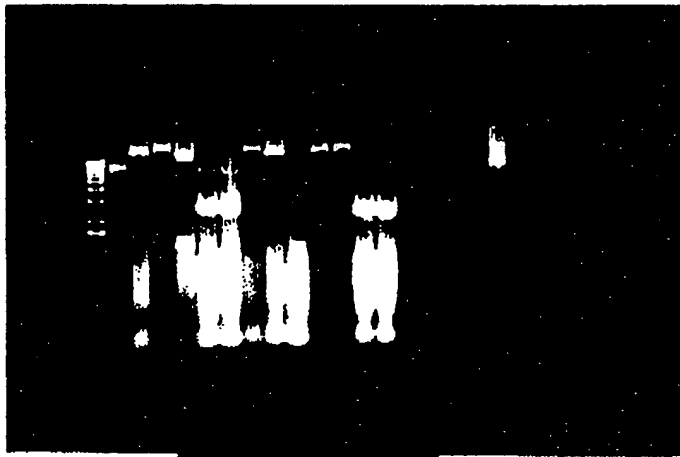
Minipreps: 8 ex (1-12).

1) ladder.

2) p223.

3) →

clones 8 ex 1-12.



1) ladder

2) pFG140 / Bam + Nde

3) p223 Bam + Nde.

(upper fragment -  
 because of incomplete  
 Nde digestion)

4) 81

5) 82

6) 86

7) 87

8) 88

9) 89

10) 810

clones (82, 86, 87,

Nde +  
 + Bam

22-141 50 SHEETS  
 22-142 100 SHEETS  
 22-144 200 SHEETS



EXHIBIT

D41

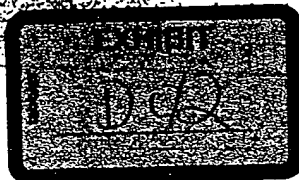
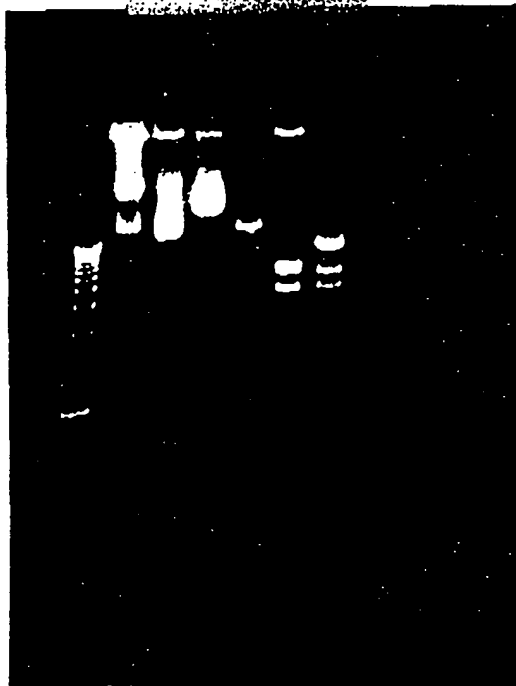
Cloning Vector  $\Rightarrow$  82 / ~~Not~~ XbaI - vector 1 fragment  
 R fragment ~ 10 kb  
 N fragment ~ 9 kb

insert p31 / EcoRI + XbaI - 1 fragment  
~~Not~~ EcoRI + XbaI  
~~Not~~ Notet + XbaI

Digestion Vector 82 / R1 XbaI 1 fr. 5 ml (0.1 / 10)  
 insert 31 / R1 XbaI 1 fr. 5 ml (0.1 / 15)  
 +  
 2 ml Buffer  
 1 ml Ligase

2 x 100 prep. of 82

- 1) Col. 82
- 2) 220
- 3) 223
- 4) 82
- 5) 110 / 110
- 6) 225 / 110 + Notet
- 7) 82



January 25, 1997.

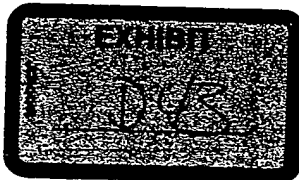
g c and gex → 100 bac. colonies / dish  
in both cages / → (defective)Transfection: 1101/1107/EGFR (0.5 r/μl)  
+ pK82 (E4→SPB) (10 r/μl)  
+ CMV/ITF (2 r/μl)

10 6cm dishes: 4 control + 6 experiments

1) control A) 8 μl (4 r) 1101/1107 (1 ml recip.)

2) control B) 8 μl 1101/1107/EGFR + 5 μl CMV/ITF →  
(4 r) (10 r)  
⇒ control see wt and toxicity of ITF.

3) exp. 8 μl (1101/1107/EGFR) ⇒ 4 r

A) + 10 μl p82 → 10 r.  
+ 3 μl CMV/ITF ⇒ 6 rB) 8 μl 1101/1107/EGFR ⇒ 4 r  
+ 5 μl p82 ⇒ 5 r  
+ 5 μl CMV/ITF ⇒ 10 rC) 8 μl 1101/1107/EGFR ⇒ 4 r  
+ 15 μl p82 ⇒ 15 r  
+ 1 μl CMV/ITF ⇒ 2 r22-141 50 SHEETS  
22-142 100 SHEETS  
22-144 200 SHEETS



Sunday, February 2, 1997

Transfection

8 large dishes.

2 - 826 + loc 2 (left-hand)

2 - 11 + loc 2 (left-hand)

2 - 1101/1107/RT - control + CMV/ITR

2 - 1101/1107/RT + p82 + CMV/ITR

400 DNA for 2 ml of precipitate - each dish

(826) 4 ml of precipitate  $\Rightarrow$

$\Rightarrow$  2 ml NEBS 2X + 100  $\mu$ l  $CaCl_2$

p loc = 80  $\mu$ l (40x)  $\checkmark$  2 ml  $H_2O$

p loc 2 = 40  $\mu$ l (40x)  $\checkmark$

(11)

p 11 = 40  $\mu$ l (40x)  $\checkmark$

p loc 2 = 40  $\mu$ l (40x)  $\checkmark$

(82 = control)  $\checkmark$  1101/1107/RT - 25  $\mu$ l (12x)

$\checkmark$  pCMV/ITR - 20  $\mu$ l (40x)

(82 = exper.)

$\checkmark$  1101/1107/RT - 25  $\mu$ l (12x)

$\checkmark$  pCMV/ITR - 20  $\mu$ l (40x)

$\checkmark$  p82 - 30  $\mu$ l (40x)

EXHIBIT

11/11

Wednesday, February 5, 1997

Transformation of *eci* with 626 + *lacZ* (Kobayashi with ITR)  
~~626~~ + *lacZ* (--- a ---)  
 626 + *lacZ* (Gard's phage...)

626 + M

2 ml HEB5 2X + 2 ml H<sub>2</sub>O + 100  $\mu$ l Gd<sub>2</sub>

✓ p626 - 100  $\mu$ l (500)

✓ pKobayashi's - 5  $\mu$ l (80)

626 + M

✓ pDUG11 - 50  $\mu$ l (500)

✓ pMoloch's - 5  $\mu$ l (80)

626 + C

✓ p626 - 100  $\mu$ l (500)

✓ pKobayashi's - 20  $\mu$ l (200)

TTC TCTAGA AGT.... ATG A GATCTAGA

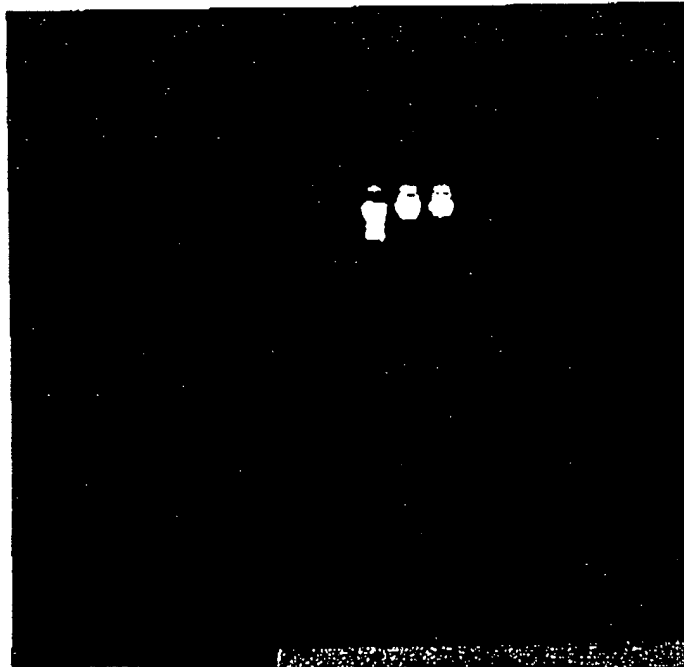
1) ladder

2) - 42 / X6

3) - 45 / X6

⇒ linear form

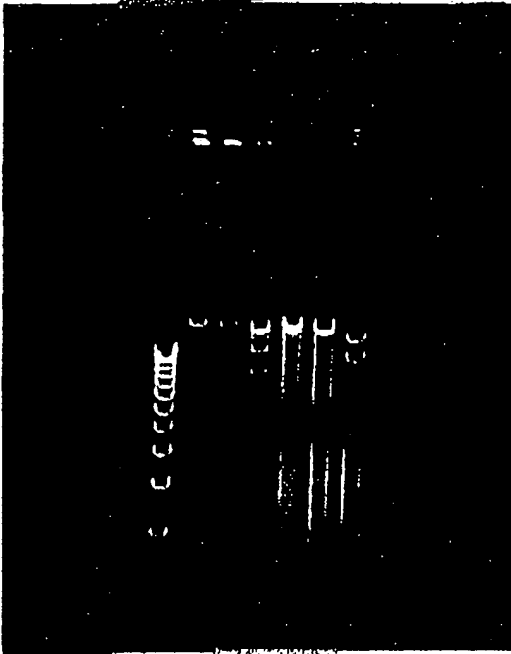
2nd X6 is the  
 plasmid is methylated!



EXHIBIT

1-2045

Sunday, February 9, 1997



Analysis of 1101/1102 (R1)  
 54  
 Pleser 54-2

1) Gdler.

2) 1101/1102

3) 1101/1102

Pec 1

4) 1101/1102

Pec 1 + Ban H1

5) 54-2

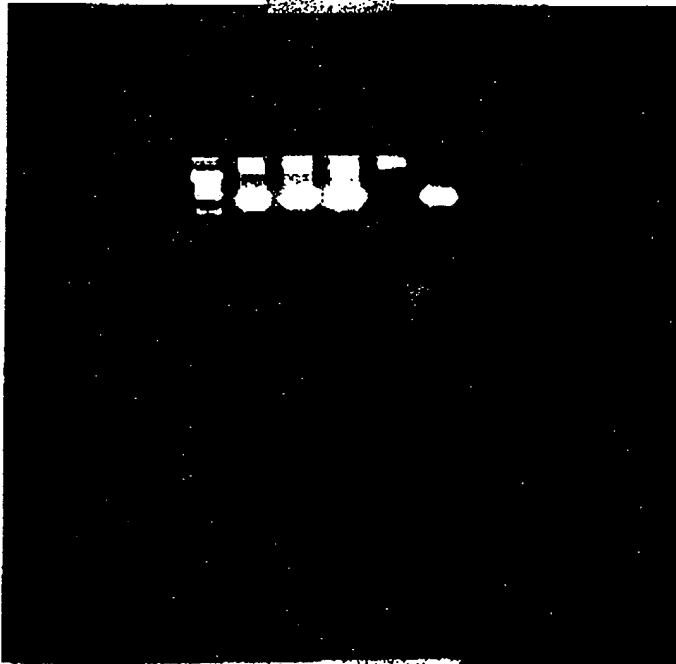
6) 54-2

Pec

7) 54-2

Pec + Ban H1

Conclusion 54-2 - wt  
 but there are  
 additional bands



PCR primers  
 (D1, R1)

1) Gdler

2) PFG 140

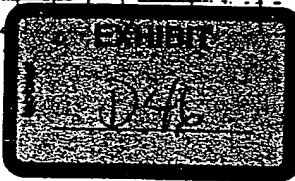
3) p82

4) p54

5) Pleser 54-2

Conclusion -

54-2 - wt 63



February 10, 1997.

Transfection of Mohr's 293/CAN/ITF with p82.

(Control) : 50  $\mu$ l of 1101/1107/EcoRI (10 $\times$ )  
 + 20  $\mu$ l pCAN/ITF (20 $\times$ )  
 + 100  $\mu$ l p82 (100 $\times$ )

3 ml H<sub>2</sub>O  
 3 ml HEBS 2X  
 300  $\mu$ l CaCl<sub>2</sub> 2.5M.

(p82) 50  $\mu$ l 1101/1107/R1.  
 + 20  $\mu$ l pCAN/ITF  
 + 100  $\mu$ l carrier DNA

February 11, 1997.

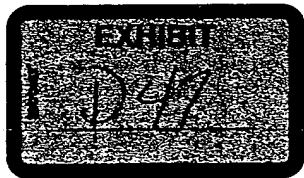
Repetition of ex. vector  $\rightarrow$  CIP  $\rightarrow$  heat inactivation

82/R1-K6 (vector) - 5  $\mu$ l -  
 81/R1-K6 (insert) - 10  $\mu$ l  
 CIP buffer - 2  $\mu$ l  
 ligase - 1  $\mu$ l.

February 14, 1997.

Transfection with 1101/1107/EcoRI + or pL2  
 p54 (L2+ADP)

|        |  |                                     |
|--------|--|-------------------------------------|
| Cont A | 10 $\mu$ l 1101/1107/EcoRI (5 $\times$ ) | + nothing                           |
| Cont B | -----                                    | + 30 $\mu$ l carrier (30 $\times$ ) |
| L2 A   | -----                                    | + 30 $\mu$ l L2 (30 $\times$ )      |
| L2 B   | -----                                    | + 30 $\mu$ l L2 (30 $\times$ )      |
| 54 A   | -----                                    | + 50 $\mu$ l 54 (25 $\times$ )      |
| 54 B   | -----                                    | + 50 $\mu$ l 54 (25 $\times$ )      |

1 ml HEBS 2X + 1 ml H<sub>2</sub>O + 100  $\mu$ l CaCl<sub>2</sub>

Cloning experiment 9 (Dx)

with new vectors 82 /  $U6 + K1$  (1)

82 /  $K1 + U6$  (2)

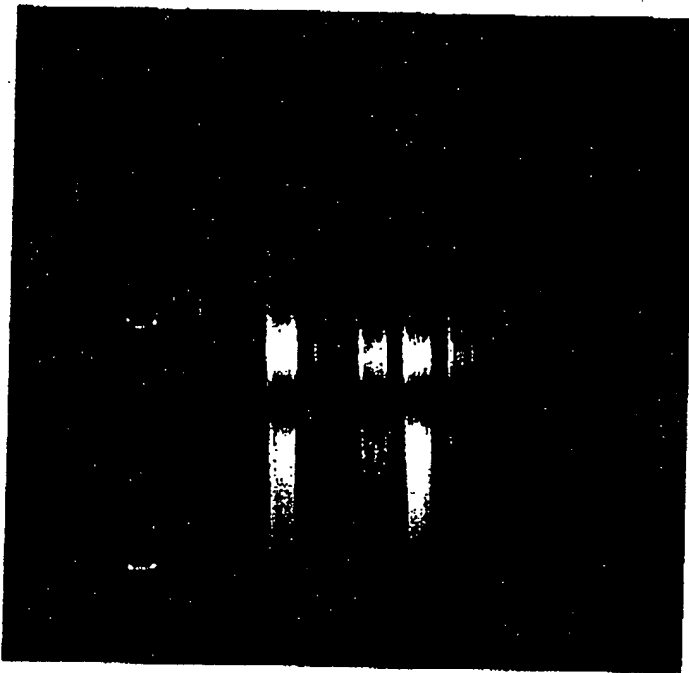
C vect 0.115 site ~ 10%

C insert 0.115 ~ ~ 9%

9 ex 1 - spl vect + spl insert

9 ex 2 - spl vect + spl insert

February 20, Thursday



Analysis of  
restriction  
pattern of  
plasmids

1) ladder 1 kb

2) 100% 1 kb

3) 1 kb

4) 54-1

5) 54-2

6) 54-3

7) 54-4

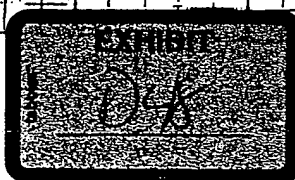
8) 54-5

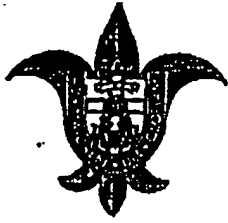
100%  
BamHI

for 54-1  
54-3  
54-4

restriction pattern

is as expected for  
recombinant virus





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Department of Molecular  
Microbiology & Immunology

Health Sciences Center  
School of Medicine

**Facsimile Transmittal**

Date: 12-3-02

From: William Wold

Phone: 314-577-8432

Fax: 314-773-3403

Deliver to: Dan Kasten

Phone: Fax: 552-76305

Total number of pages including this page: 12

Message:

Kostya's lab notes.

KOI is made  
2/20/97

2-20-97

50 SHEETS  
100 SHEETS  
200 SHEETS



PCR of Koi prep  
from previous plate  
primers left 1 and right 7

- 1) ladder 1ul
- 2) pG140 (1150g)
- 3) pS4
- 4) Koi
- 5-9) -
- S4 1-5  
respectively

viruses S4-2  
and S4-5  
contain both wt  
and recombinant ADP

there is but as it is wt is more!

S4-1  
S4-3  
S4-4 } - recombinants!

02-25-97

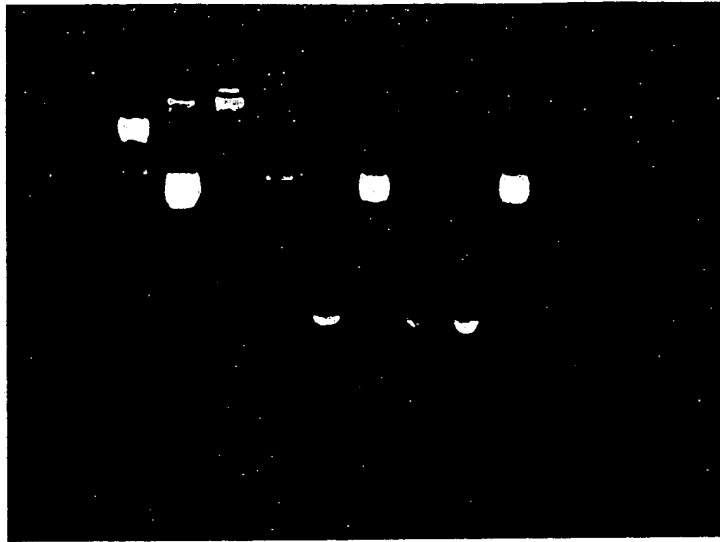
9a. → 2 time purified from gel vector.  
p82 / R1 & Co. } A fr.  
insert p31 / R1 & Co.

vector 10ul 82 + 8ul H<sub>2</sub>O + 2ul buffer

vector lig 10ul 82 + 8ul H<sub>2</sub>O + 2ul buffer + 1ul ligase

vector + insert 10ul 82 + 8ul insert + 2ul buffer + 1ul ligase

2.20.91



PCR of Unt prgs  
from previous picture.  
primers 10D1 and 10D7.

- 1) Gddex 1ul.
- 2) pFG140 (dl sos)
- 3) p54
- 4) Mock
- 5 - 9) -
- 54 1 - 5
- respectively.

viruses 54-2  
and 54-5  
contain both wt  
and recombinant ADP!

there is band as in wt in movie!

54-1 }  
54-3 } - recombinants!  
54-4 }

82-25-97

9ex → 2 time purified then gel vector.  
p82 / R1 x62.  
insert p31 / R1 x62 } 11 fr.

vector 10 μl 82 + 8 μl H<sub>2</sub>O + 2 μl buffer

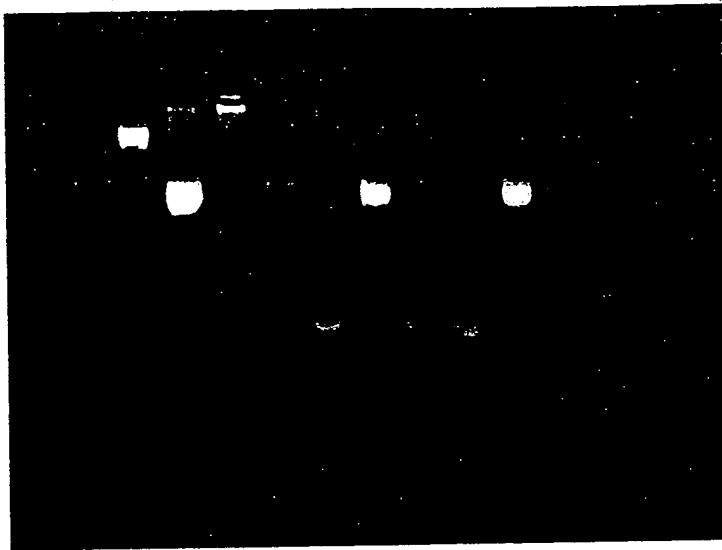
vector+lig 10 μl 82 + 8 μl H<sub>2</sub>O + 2 μl buffer + 1 μl ligase

vector+insert 10 μl 82 + 8 μl insert + 2 μl buffer + 1 μl ligase  
~~in 10 μl~~



2-20-97

22-141 50 SHEETS  
22-142 100 SHEETS  
22-144 200 SHEETS



PCR of Mint frags  
from previous picture.  
primers 1eD 1 and 1eD 7.

- 1) ladder 1ul.
- 2) pFG140 (dl 309)
- 3) p54
- 4) mock
- 5-9) -
- 54 1-5
- respectively.

viruses 54-2  
and 54-5  
contain both wt  
and recombinant ADP!

there is band as in wt in movie!

54-1  
54-3  
54-4 } - recombinants!

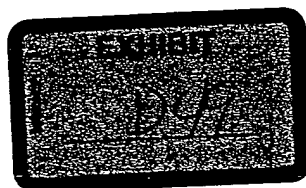
82-25-97

9ex. → 2 time purified thru gel vector.  
p82 / R1 x62.  
insert p31 / R1 x62 } 1 fr.

vector 10ul 82 + 8ul H<sub>2</sub>O + 2ul buffer

vector + lig 10ul 82 + 8ul H<sub>2</sub>O + 2ul buffer + 1ul ligase

vector + insert 10ul 82 + 8ul insert + 2ul buffer + 1ul ligase  
~~vector + insert~~



02-25-97

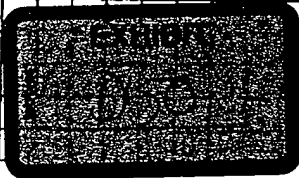
54-10 plaques - phage type that I like  
 WT + T<sub>EX</sub> or T<sub>EX</sub> with high MOI?

Sex - try 80% - 16?

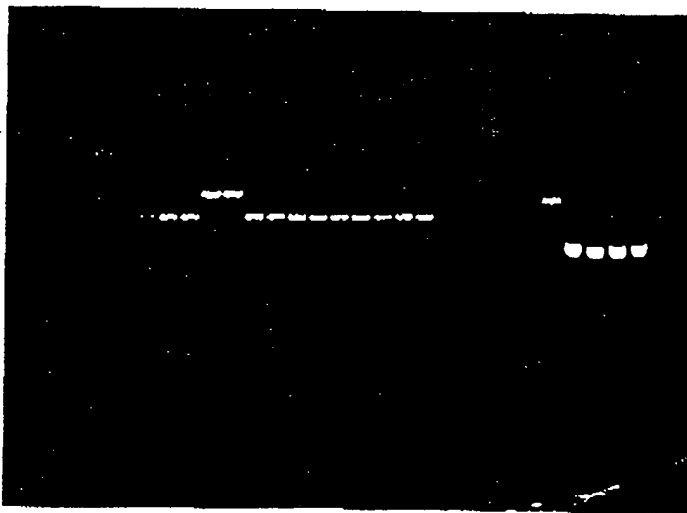
03-05-97 Wednesday.

Clay Experiment 10 ex  $\Rightarrow$  pL2 + E3 from gp13 (p31)  
 (sites EcoRI + NdeI).

11 ex  $\Rightarrow$  pL2 + E3 from pE3X66 + ADP  
 (P42)  
 (sites  $\Rightarrow$  EcoRI + NdeI).



PCR analysis  
 of plaques p82  
 and p54.



1) Ladder

2) Blank

3) Mock

4) 100/1100

5) PF6140

6) p225

7) p82

8) 100/1100

9) 821

10) 823

11) 824

12) 825

13) 826

14) 827

15) 8210

16) 8211

primers  
 10, 11

17) - Ladder

18) - Blank

19) - Mock

20) 100/1100

21) PF40

22) 54

23) 546

24) 548

25) 549

26) 5410.

primers 1, 11

p82 + 1121/1107 | Erol | plagues!

AC wt1 - medium size } wt control  
AC wt2 - large size }

pc wt2 - large size

transfection  
from 01-25.

A. 1, 2 - medium.

B 3 - medium.

→ y - small

§. - medim.

B. 6, 7, 8- and 9-

g - small

C - 10. medium.

1.1 - 3 mol.

12, 13, 14 - med. dis.

(15), (16), (17) - red in

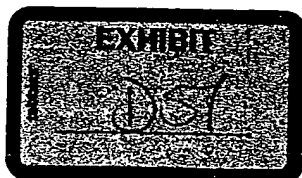
Does it not.

7 transfer from  
from 02-02

Fungal contamination in plate wells

827  
and 547 > lost due to Prologe of Eppendorf  
tubes.

⇒ no recombinants with p82 according to PCR results.  
p54 = ? ⇒ no product of PCR, just RNA.



05-06-97

Ligation 10 ex, 11 ex.

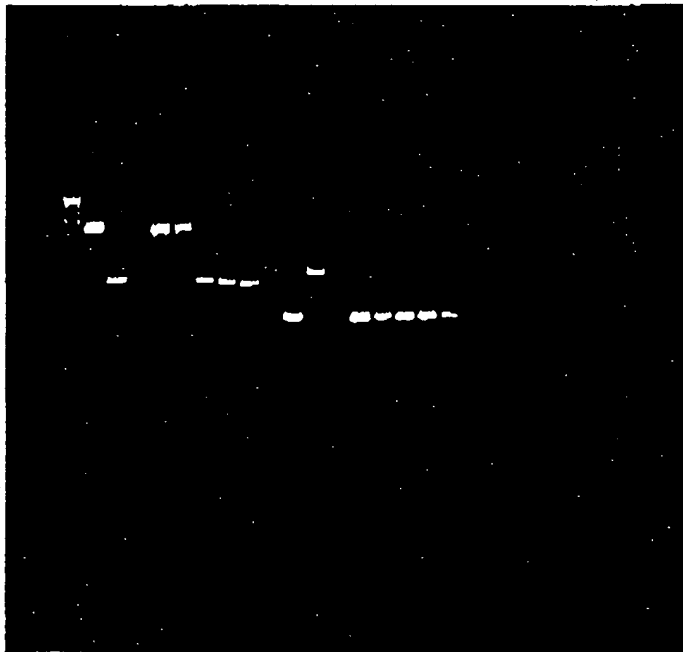
5  $\mu$ l vector (L2/ENAH)

→ V

+ ligase → VL

+ 10  $\mu$ l  
31 ENH → 10 ex L+ 15  $\mu$ l  
31 ENH → 10 ex H+ 15  $\mu$ l  
42 EN → 11 ex

PCR Analysis of plaques



1) ladder

2) 140

3) 54

4) mock

5) WT 1.7/1.7

6) 546

7) 548

8) 549

9) 540

10) blank

11) p82

12) p82

13) mock

14) WT 1.7/1.7

15) 828

16) 825

17) 827

18) 829

548, 549, 540

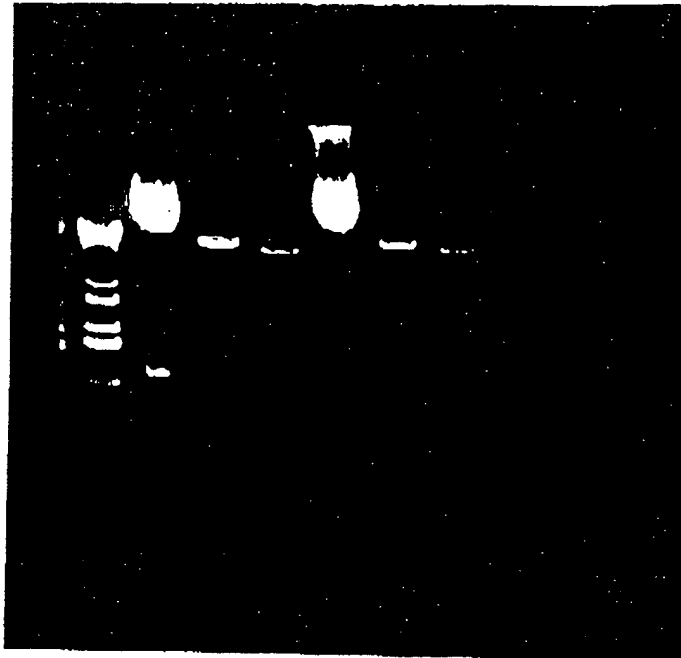
+ recombinants

with p82 all  
plaques are WT.

EXHIBIT

102

22-141 50 SHEETS  
22-142 100 SHEETS  
22-144 200 SHEETS



- 1) Ladder
  - 2) pL2
  - 3) p31
  - 4) p42
  - 5) pL2
  - 6) p31
  - 7) p42
- { EcoRI +  
 Nde,  
 buffer MC  
 { EcoRI +  
 + Nde,  
 buffer )

03-07-97

Transfection p82 into 293/TTR.

12 dishes — 6 - control ( 3 ml of precip. ) — 6 - exp. DNA  
 6 - experiment ( ————— )

(control) 25  $\mu$ l 1101/1107/R1 (5r) 1.5 ml LIEBSON  
 + 5  $\mu$ l pCMV TTR (10r) 1.5 ml H<sub>2</sub>O  
 + 30  $\mu$ l GoldTiter. (~300) 150  $\mu$ l CaCl<sub>2</sub>  
 DNA.

(exp. p82) 25  $\mu$ l viral DNA  
 + 5  $\mu$ l pCMV TTR  
 + 30  $\mu$ l p82

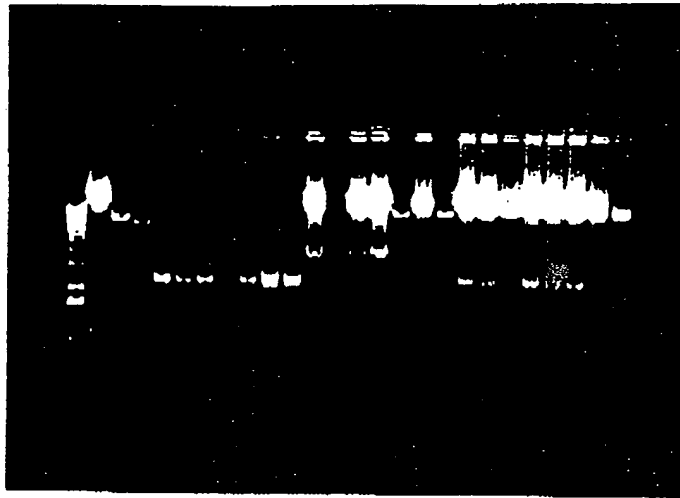


03-10-97  
Cloning 10ex, 41 ex.

1 - ladder  
2 - p12  
3 - p31  
4 - p42  
5 - 10L-1  
6 - 10L-2  
7 - 10L-3  
8 - 10L-4  
9 - 10L-5  
10 - 10L-6  
11 - 10L-7

12 - 10H-1  
13 - 10H-2  
14 - 10H-4  
15 - 10H-5  
16 - 10H-6  
17 - 10H-7  
18 - 10H-8

19 - 11-1  
20 - 11-2  
21 - 11-3  
22 - 11-4  
23 - 11-5  
24 - 11-6  
25 - 11-7  
26 - 11-8



19 - all clones - rect 1

10H - all clones - recant hump  
lower band was visible in  
all clones in short run

Take 11 and 10  
for GSCC maps.

Enoki + Mest

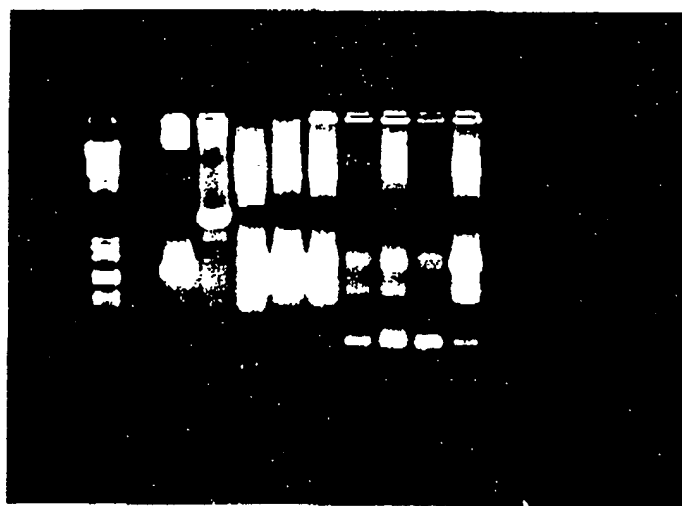
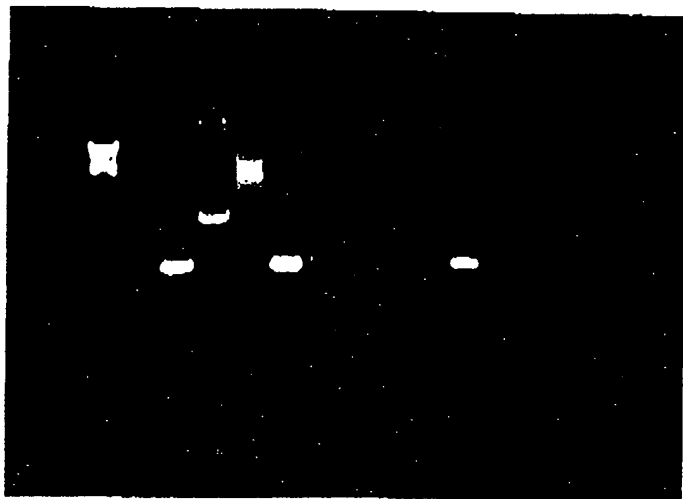
EXHIBIT

10/24

03-12-97

lint traps of small amount (plate) of 293/TTC  
infected with slas growing primers p82(?)  
PCR, primers 10, 11.

22-141 50 SHEETS  
22-142 100 SHEETS  
22-143 200 SHEETS

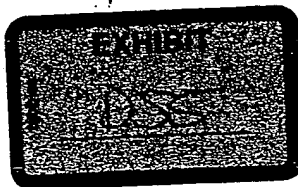


- 1) ladder.
- 2) blank (H<sub>2</sub>O)
- 3) pR6100.
- 4) p82
- 5) mock.
- 6) wt.
- 7) 8212
- 8) 8214
- 9) 8215
- 10) 8216
- 11) 82-17

Conclusions:

blank. clean.

in 82-12, 82-17 -  
band, corresponding to wt.  
and weak additional  
band corresp. to Rec.  
(does not present in wt!!!)  
Something of a bit smaller  
than present in mock



3-17-97

PCR analysis of p82 resulting plaques / primers

LD 8, LD 9 -

- SPB - promoter  
specific.

1) ladder.

2) blank.

3) pG140.

4) p82

5) mock.

6) wt 1.01/110X

7) 821

8) 822

9) 823

10) 824

11) 825

12) 826

13) 827

14) 828

15) 829

16) 8210

17) 8211

18) 8212

19) 8213

20) 8214

21) 8215

22) 8216

On cloning, there is SPB in 295 cells,  
viruses are rather wt than  
recombinants. need to use

(LD 8, LD 9) combination of primers / SPB specific



3-18-97

Sequencing

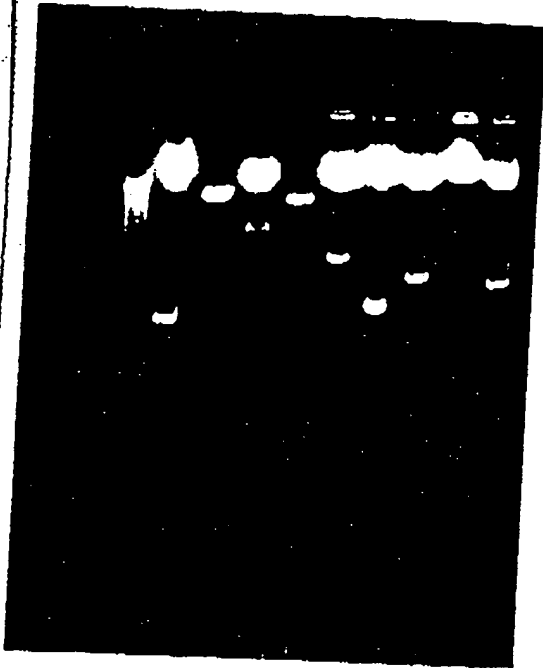
K) 9 ( p21, primer K) 10)

K) 10 ( p21, primer K) 11)

K) 11 ( p111, primer K) 4)

K) 12 ( p111, primer K) 5)

Restriction pattern of C5cl frags. of p101, p111

22-141 50 SHEETS  
22-142 100 SHEETS  
22-144 200 SHEETS

- 1) 1 kb ladder
- 2) L2 / R1 + NdeI
- 3) p31 / R1 + NdeI
- 4) p101 / R1 + NdeI
- 5) p42 / R1 + NdeI
- 6) p111 / R1 + NdeI
- 7) L2 / PaeI + SpeI
- 8) p101 / PaeI + SpeI
- 9) L2 / SpeI + XbaI
- 10) p111 / SpeI + XbaI



3-21-97

Analysis of sequences K) 9, 10, 11, 12.

Everything is fine with p111. As predicted.

p21 - sequence with all primer is fine.

- sequence with K) 10 primer shows that the *E. coli* sequence is going after Ad at 35858 and up to the end of sequence (approx 800 bp) → see

⇒ p626 and p82 are not resolvable in virus  
Need to check p34611 for the same mutation.

EXHIBIT

1058

cases 403  
3/27/97

3-20-97 Regues. (experiments of 02/05-02/02)

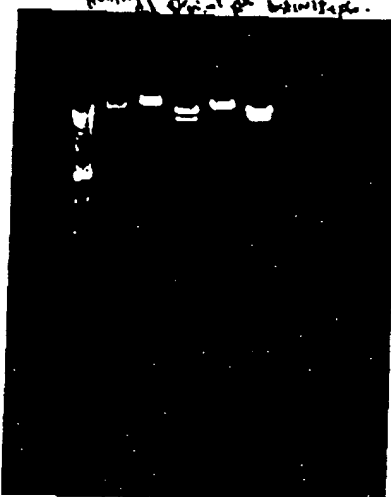
626M 1-12 (12)

626C 1-6 (6)

11M 1-4 (4)

3-25-97

32-41 50 SHEETS  
32-42 100 SHEETS  
32-43 200 SHEETS



1) Collier 148

buffer-M.

2) 1101/1107 DNA / EcoRI - SpeI

3) p82

4) p82 / Bst 1107 I

5) p82 / Xba I

6) p82 / Bst 1107 I + Xba I

There is Bst 1107 I site at Ad 29012!

2nd is after SpB promoter

3-27-97

Transfection 293 with p101, p111 + 1101/1107 / EcoRI + SpeI  
8X Gen dishes.

3 dishes 101 (for 1 dish) 200  $\mu$ l NEBS 2X + 200  $\mu$ l H<sub>2</sub>O + 10  $\mu$ l 101

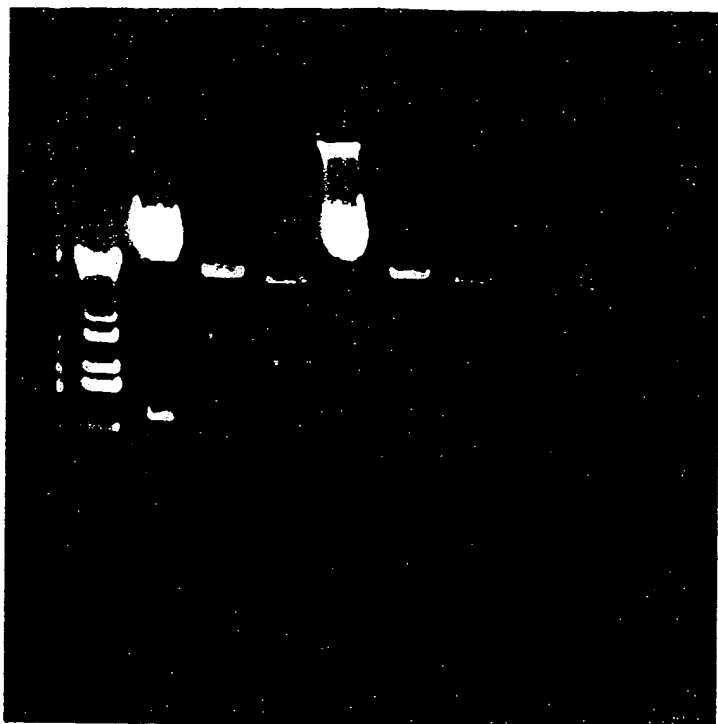
3 dishes 111 (for 1 dish) 200  $\mu$ l NEBS 2X + 200  $\mu$ l H<sub>2</sub>O + 10  $\mu$ l 111

2 dishes control (for 1 dish) 200  $\mu$ l NEBS 2X + 200  $\mu$ l H<sub>2</sub>O

+ 10  $\mu$ l 1101/1107 + 25  $\mu$ l GCl<sub>2</sub>.



22-141 50 SHEETS  
22-142 100 SHEETS  
22-144 200 SHEETS



- 1) ladder
  - 2) pL2
  - 3) p31
  - 4) p42
  - 5) pL2
  - 6) p31
  - 7) p42
- { EcoRI +  
 Nde,  
 buffer MC  
 { EcoRI +  
 + Nde,  
 buffer D

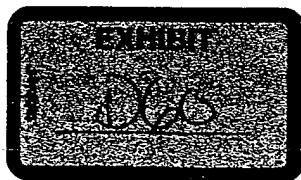
03-07-97

Transfection p82 into 293/TTR.

12 dishes — 6 - control (3ml of precip) — 60% DNA  
 6 - experiment (— — — — —)

(control) 25µl 1101/1107/R1 (5r) 1.5ml LIEBS 2X  
 + 5µl penicillin (100) 1.3ml H<sub>2</sub>O.  
 + 30µl glutaric. (~300) 150µl CaCl<sub>2</sub>  
 DNA.

(cover p82) 25µl viral DNA.  
 + 5µl penicillin  
 + 30µl p82



03410-97

Cloning 10 ex, 41 ex.

1 - ladder

2 - pL2 7

3 - p31

4 - p42

5 - 10L-1

6 - 10L-2

7 - 10L-3

8 - 10L-4

9 - 10L-5

10 - 10C-6

11 - 10C-7

12 - 10H-1

13 - 10H-2

14 - 10H-4

15 - 10H-5

16 - 10H-6

17 - 10H-7

18 - 10H-8

19 - 11-1

20 - 11-2

21 - 11-3

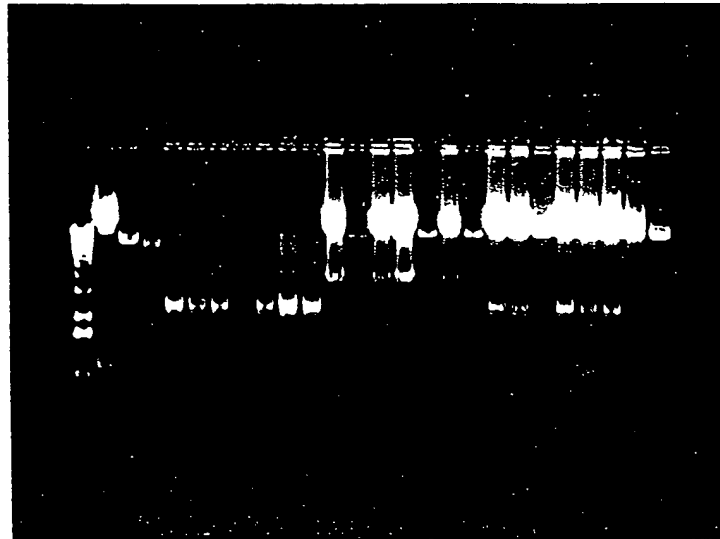
22 - 11-4

23 - 11-5

24 - 11-6

25 - 11-7

26 - 11-8



19 - all clones - rect 15.

10H - all clones - recombinate

lower band was visible in  
all clones in short run.

Take 11 and 101  
for C&C prep.

Excl + 10 ex

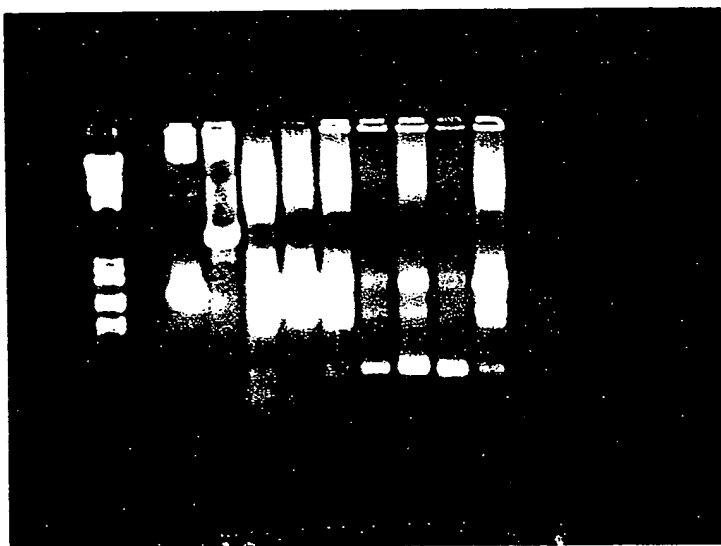
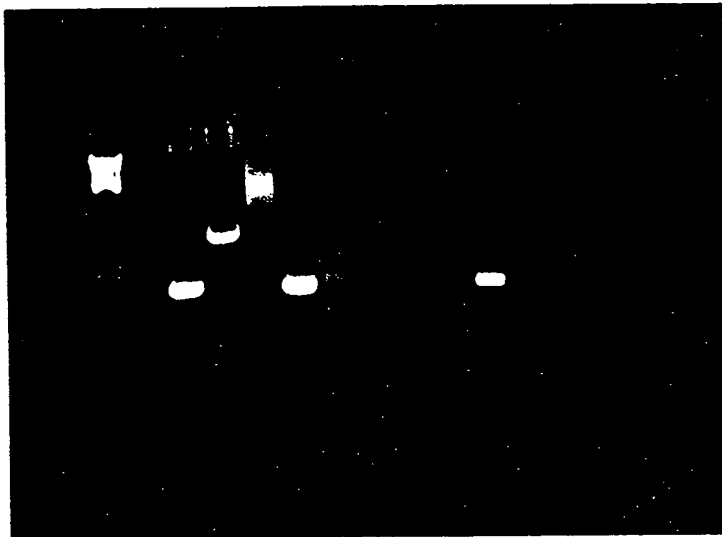
EXHIBIT

D&L

03-12-97

Hint preps of small amount (plate) of 293/TTF  
infected with slow growing viruses p82(?).  
PCR, primers 10, 11.

22-141 50 SHEETS  
22-142 100 SHEETS  
22-143 500 SHEETS



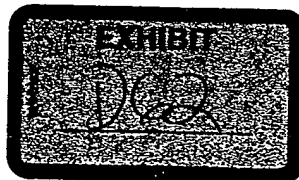
- 1) ladder.
- 2) Blank (H<sub>2</sub>O)
- 3) pF6140.
- 4) p82
- 5) mock.
- 6) wt.
- 7) 82-12
- 8) 82-14
- 9) 82-15
- 10) 82-16
- 11) 82-17.

Conclusions:

Blank - clean.

in 82-12, 82-17 -  
band, corresponding to wt.  
and weak additional  
band corresp. to rec.

(does not present in mock!!!  
Something of a bit smaller  
MW present in mock

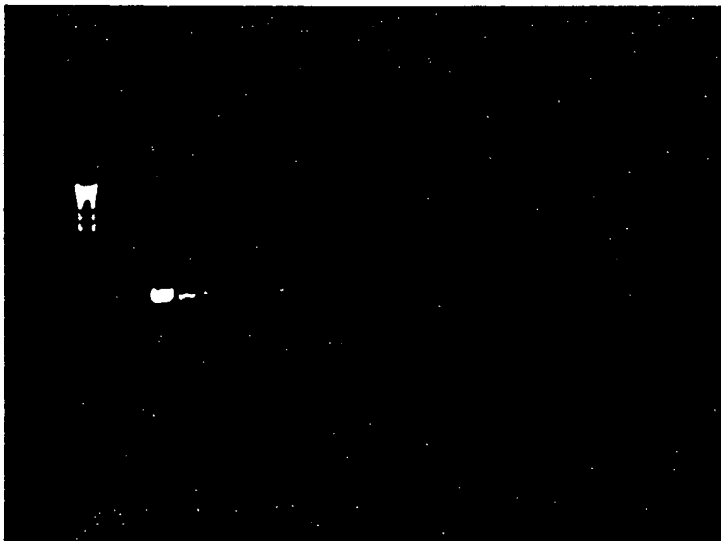
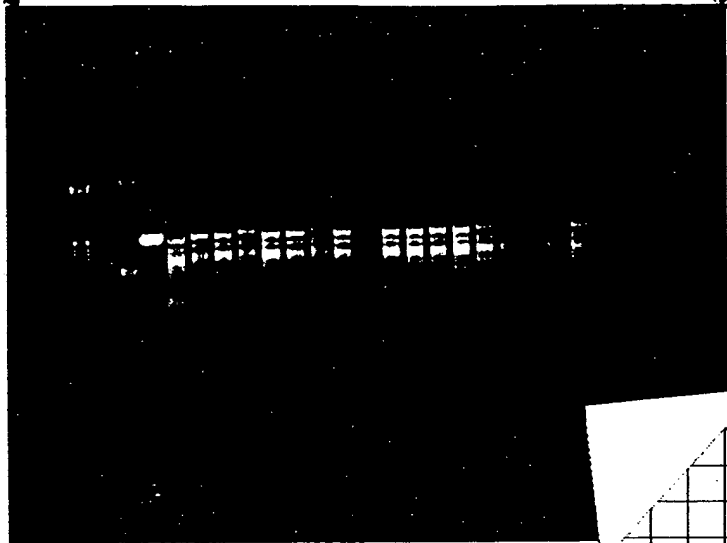


3-17-97

PCR analysis of p82 resulting plaques (primers

wd 8, wd 9 -

- SPB - promoter -  
specific).



1) ladder

2) blank

3) pF6140

4) p82

5) mock

6) wt 1101/1107

7) 821

8) 822

9) 823

10) 824

11) 825

12) 826

13) 827

14) 828

15) 829

16) 8210

17) 8211

18) 8212

19) 8214

20) 8215

21) 8216

22) 8217

Conclusion: there is SPB in 293 cells,  
viruses are rather wt than  
recombinants, need to use

(12) 8, (14) 11 combination of primers (9 - SPB specific)  
(11 - AI specific)

3-18-97

Sequencing

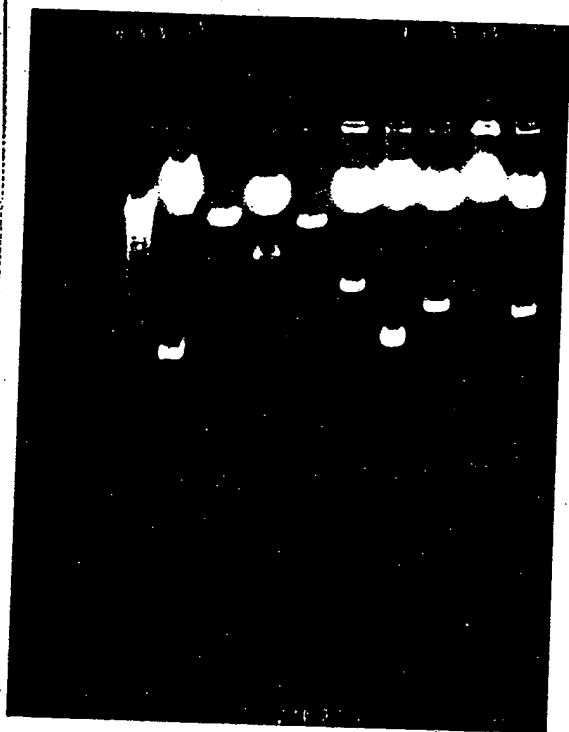
KD 9 ( p 21 , primer KD10 )

KD 10 ( p 21 , primer KD11 )

KD 11 ( p III , primer KD4 )

KD 12 ( p III , primer KD5 )

Restriction pattern of Csl preps. of p101, p111.



- 1) sub Padder
- 2) L2 / R1 + NdeI
- 3) p31 / R1 + NdeI
- 4) p101 / R1 + NdeI
- 5) p42 / R1 + NdeI
- 6) p111 / R1 + NdeI
- 7) L2 / PacI + SpeI
- 8) p101 / PacI + SpeI
- 9) L2 / SpeI + XbaI
- 10) p111 / SpeI + XbaI

3-21-97

Analysis of sequences KD 9, 10, 11, 12.

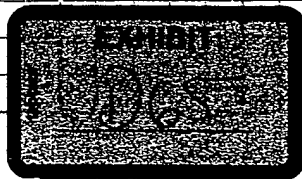
Everything is fine with p111. As predicted.

p21 - sequence with all primer is fine.

- sequence with KD 10 primed shows that the *E. coli* sequence is going after Ad nt 35858 and up to the end of sequence (~500 bp).  $\Rightarrow$  see next page



⇒ p626 and p82 are not responsible for virus.  
Need to check p34611 for the same mutation.



3-20-97 Regues. (experiments of 02/05  
02/02)

626M 1-12 (12)

626c 1-6 (6)

11M 1-4 (4)

3-25-97

1) linker 1kb

buffer-ME.

2) 1101/1107 DNA / EcoRI + SpeI

3) p82

4) p82 / Bst 1107 I

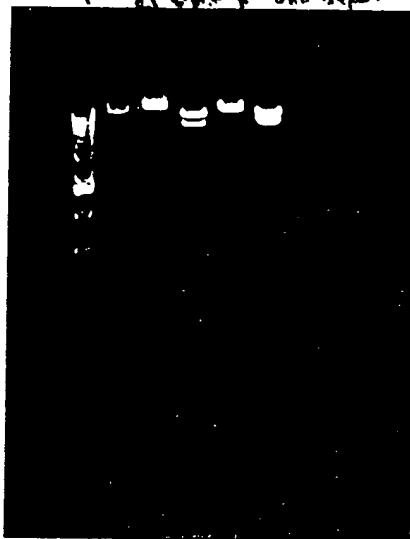
5) p82 / XbaI

6) p82 / Bst 1107 I + XbaI.

There is Bst 1107 I site in p82  
at Ad 29012!

2nd is after SOB promoter!

22-141 50 SHEETS  
22-142 100 SHEETS  
22-144 200 SHEETS



3-27-97

Transfection 293 with p101, p111 + 1101/1107 / EcoRI + SpeI  
8X Gen dishes.

3 dishes 101 (for 1 dish) 200  $\mu$ l HEBS 2X + 200  $\mu$ l H<sub>2</sub>O + 10  $\mu$ l 101

3 dishes 111 (for 1 dish) 200  $\mu$ l HEBS 2X + 200  $\mu$ l H<sub>2</sub>O + 10  $\mu$ l 111

2 dishes - control (for 1 dish) 200  $\mu$ l HEBS 2X + 200  $\mu$ l H<sub>2</sub>O

+ 10  $\mu$ l 1101/1107 + 25  $\mu$ l GCl<sub>2</sub>.



22-141 50 SHEETS  
22-142 100 SHEETS  
22-144 200 SHEETS



4/7/97

Plaque assay of 544 on 253

day  
(6)

309 - ( $\sim 2$ ) - plaques are confluent (too many plaques per dish)  
(see slide)

1101/1107 ( $\sim 2$ ) - Same as with 309.  
(CsCl and crude ligate)

544 (titer  $\sim 2$  is about 2 orders of magnitude lower than in wt/309, 401, plaques appeared  $\sim 1$  day later than in wt ( $\sim$  day 5) and are a bit smaller.

(day 16 size  $\sim 8$  mm)

7001 (CsCl) (titer is 1-2 orders of m. lower than predicted for wt)  
 $\sim 2$   
plaques are tiny (day 16  $\sim 1-2$  mm diameter)

4/8/97

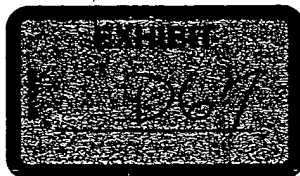
Ligation

12ex 1) 82 (10  $\mu$ l) + PCR product ITR (10  $\mu$ l)  
bst (part) + Kbs from 140 (49, 16, 17)

2) 87 (5  $\mu$ l) +

4/16/97

Ligation 12ex: vector 87/Kbs + bst (part) + CIP  
82/Kbs + bst + CIP (part) upper fragment.  
82/Kbs + bst + CIP lower fragment



4/17/97 Sequencing.

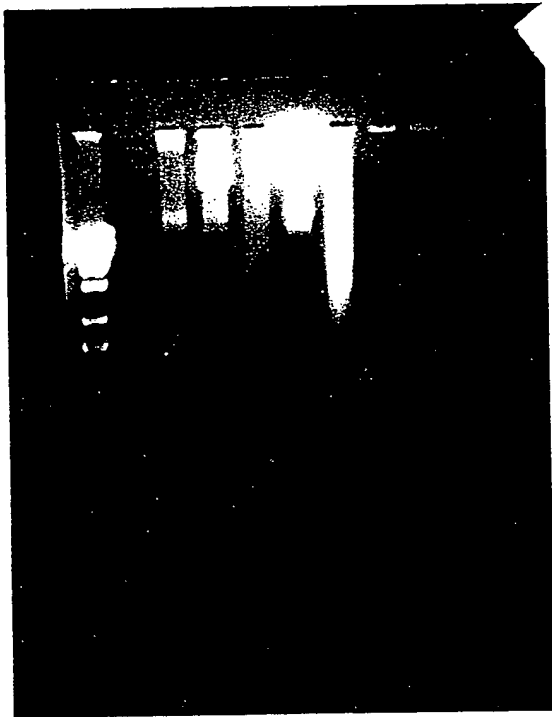
kd (14) - (pB4G11, primer kd 10)

kd (15) - (pB4G10, primer kd 10)

kd (16) - Mippen's Gel 4 large plasmid, primer kd 16

kd (17) - p82, primer kd 10

kd (18) - p111, primer kd 10.



that prep of 101-1  
and 111-1.

PCR, primers 1,5.

1) ladder.

2) blank

3) pB61wo.

4) p101.

5) 101-1

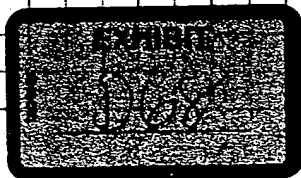
6) p 111

7) 111-1

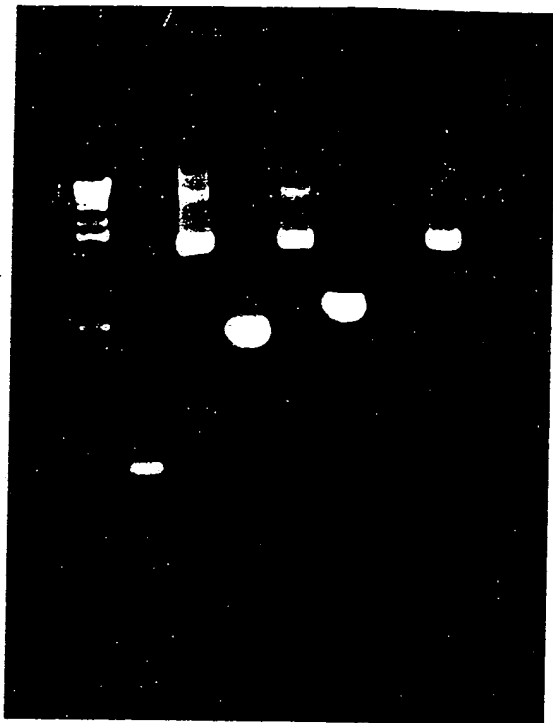
p101, p111, <sup>(111-1)</sup> PCR didn't  
work.

for 101-1 product is  
as expected for 111

Mixed tubes 10-1, 11-1  $\Rightarrow$  or what?



22-141 50 SHEETS  
22-142 100 SHEETS  
22-144 200 SHEETS



PCR, primers 1, 7.

- 1) Ladder
- 2) Blank
- 3) 140.
- 4) 54
- 5) p101
- 6) p111.
- 7) 101-1
- 8) 111-1.

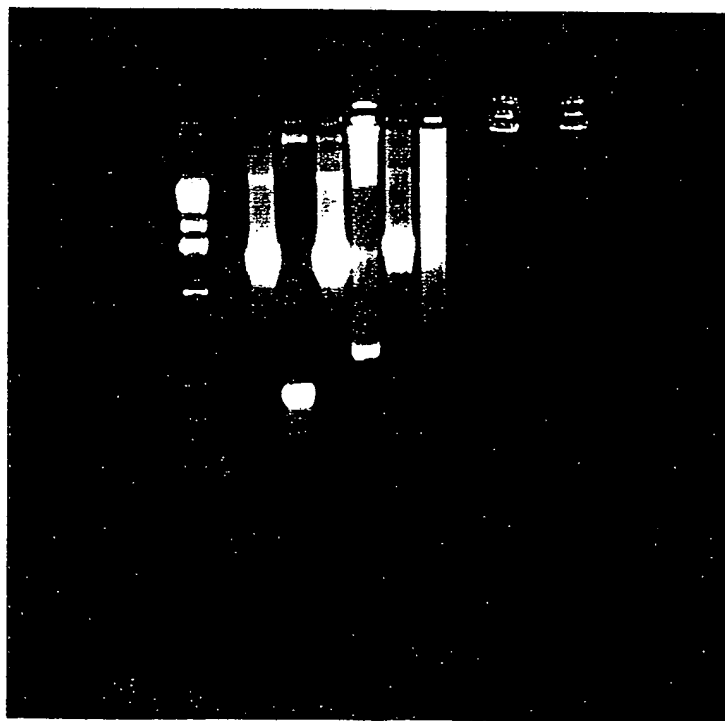
in 111-1 band is as h wt  
(or 101)

in 101-1 band is higher  
than wt.

4/21/97

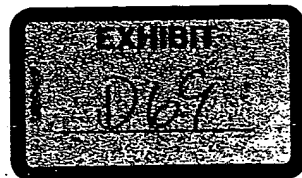
PCR, primers 1, 7.

- 1) Ladder.
- 2) Blank
- 3) p140.
- 4) p54
- 5) p101
- 6) p111
- 7) 101-1
- 8) 111-1.



Conclusion:  
this time everything  
is as expected.

- 9) 111-2
  - 10) 111-3
  - 11) 111-4
  - 12) 101-1
- } plagues.



1/22

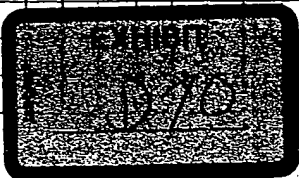
## Sequencing

- W) 19 - Cus's pED vector, primer CFC II 6
- W) 20 - pB4G10, primer KD10.
- W) 21 - p82, primer W) 16
- W) 22 - p82, primer W) 17
- W) 23 - p21, primer W) 16
- W) 24 - p21, primer W) 17

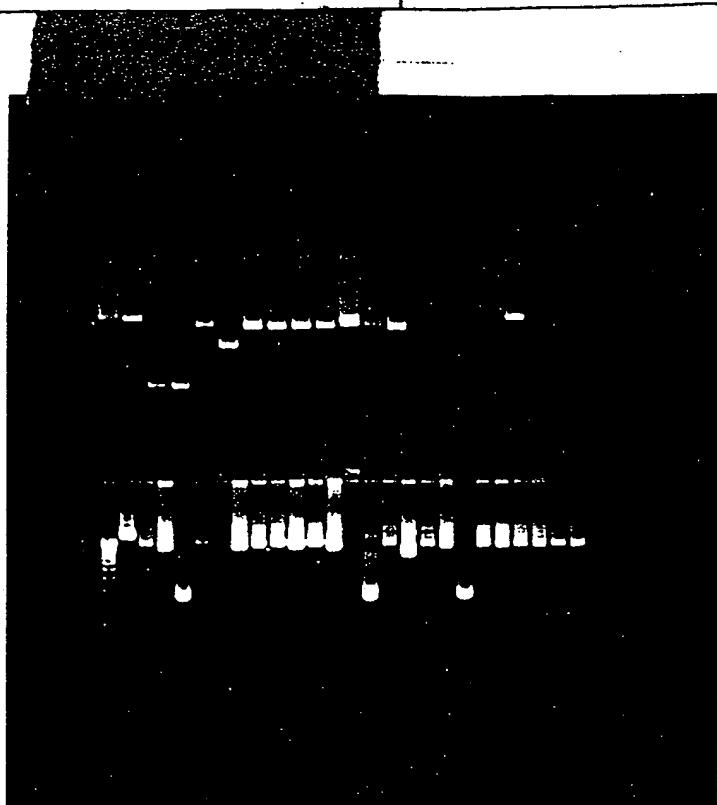
## Results of previous sequencing

- W) 14 (pB4G11, primer KD10) - sequence terminates on same point where the sequences start in p21.
- W) 15 (pB4G10, primer W) 16) - same as ↑  
primer KD16)
- W) 16 (Mippers large GAL4 plasmid) - same as ↑  
but there is some sequence which is what?
- W) 17 (p82, primer W) 16) - sequence of 30-35 bp, need to repeat with W) 16 primer.
- W) 18 (p111, primer W) 16) - sequence is perfect as predicted.

Sequences 19-24 - early termination everywhere  
⇒ new protocol doesn't work.



22-141 50 SHEETS  
22-142 100 SHEETS  
22-144 200 SHEETS

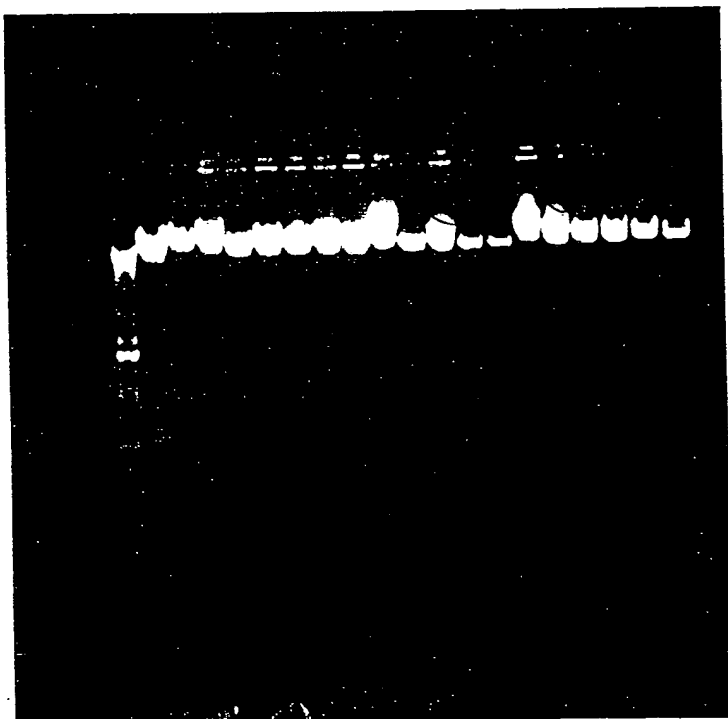
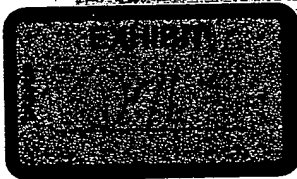


mini preps of 12 ex.

- 1) Ladder,
- 2) p82
- 3) 87-1
- 4) 87-2
- 5) 87-3
- 6) 824 ex1 (1-9)
- 7) 824 ex2 (1-12)
- 8) ↓
- 9) ↓

- 1) Ladder.
- 2) p82.
- 3) 82 L ex1 (1-12)
- 4) 82 L ex2 (1-12)

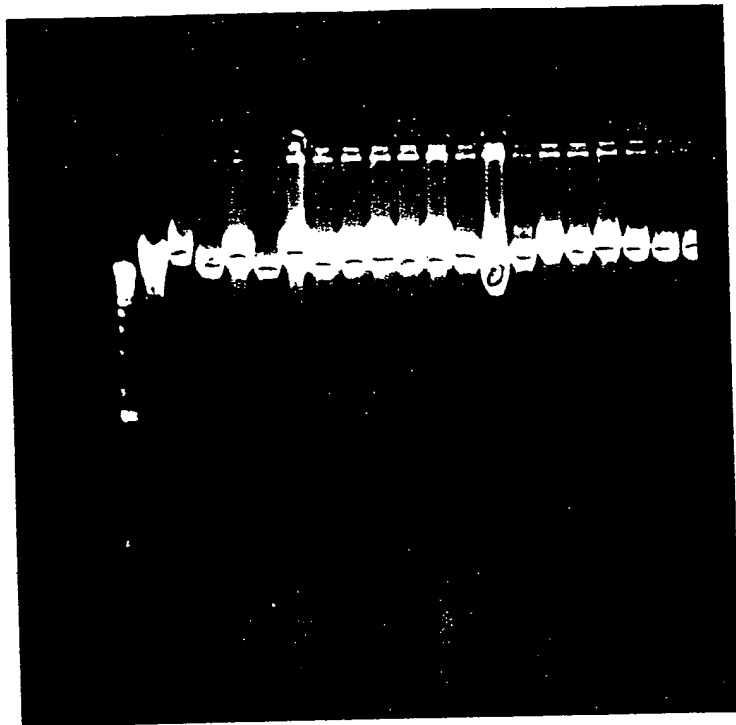
4/23 Restriction analysis of clones 12 ex



- 9) Ladder
- 1) p82 / R1
- 3) p82 / X63
- 4) 871
- 5) 824H
- 6) 824B
- 7) 8244-4
- 8) 8245
- 9) 416
- 10) 417
- 11) 418
- 12) 419
- 13) 42-1
- 14) 42-4
- 15) 42-5
- 16) 42-7
- 17) 42-8
- 18) 42-9
- 19) 42-10
- 20) 42-12

Eco RI

Conclusions: 82/121 - partial digestion.  
clones - no small bands.  
2 possibly - recombinants.  
Marked bands have lower MW than p82/X63



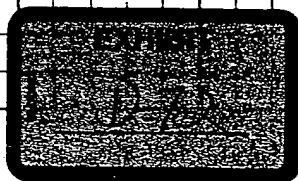
- 1) ~~adder~~
- 2) p82/R1
- 3) p82/X6
- 4) L1-1
- 5) L1-2
- 6) L1-4
- 7) L1-6
- 8) L1-7
- 9) L1-8
- 10) L1-9
- 11) L1-10
- 12) L1-11
- 13) L2-2
- 14) L2-3
- 15) L2-4
- 16) L2-5
- 17) L2-7
- 18) L2-8
- 19) L2-9
- 20) L2-10
- 21) L2-11
- 22) L2-12

Conclusion: p82 - partial  
R1 digestion  
clones - no small bands.

L2-3 - exclude from further analysis.

Tag NdeI - XbaI ~~hydro~~ digestion.

correct 5 kb fragment in rec,  
6 kb fragment in 82 (wt)

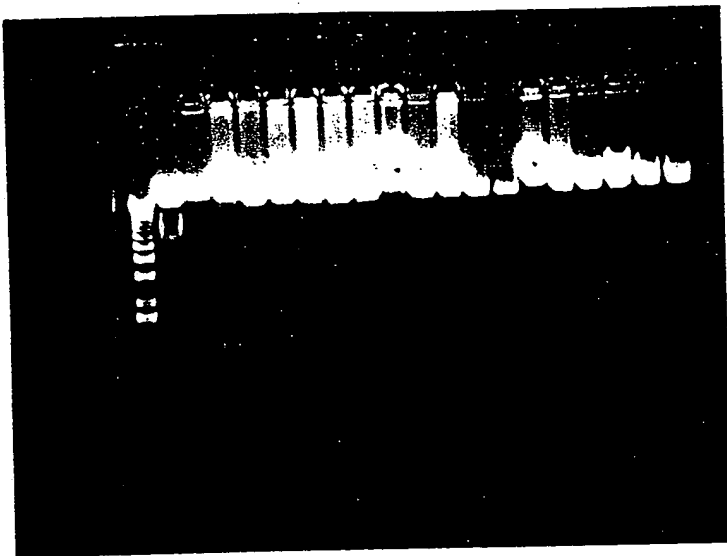




4/24

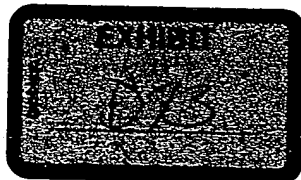
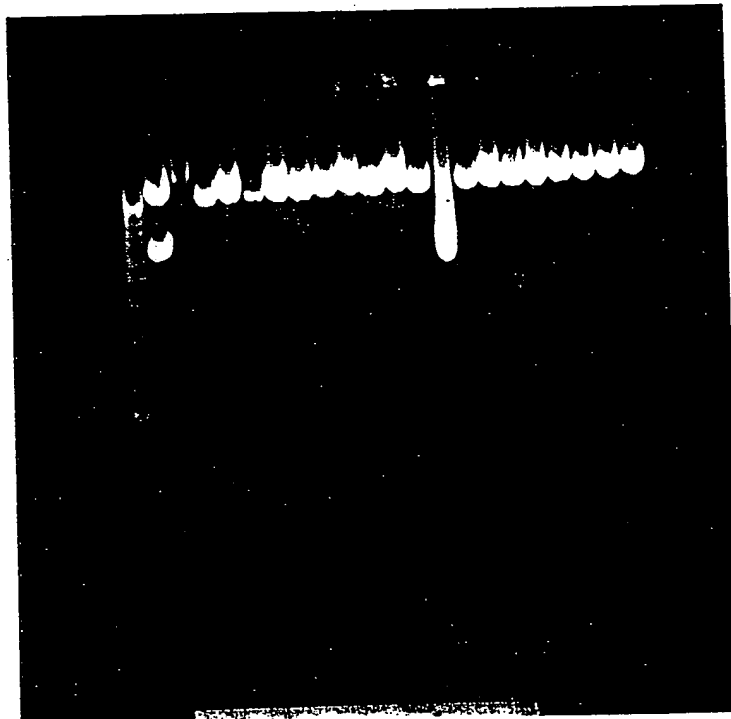
Same as previous 2  
pictures,

- 1) ladder
  - 2) p82 / Xba + Nde
  - 3) p82 / Nde.
  - 4) clones / Xba + Nde
- ↓

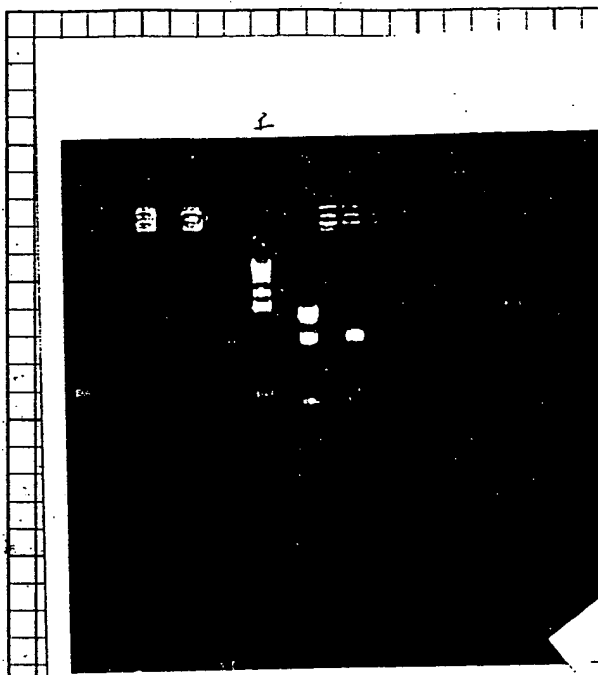


Conclusion → all these  
clones are junk,  
start cloning at 170  
into 223 where Bst<sub>III</sub>  
is unique, then put  
into resulting plasmid  
ben-Nde fragment from  
p2G140 (uniqueness  
again).

Probably, most of them  
are dt Bst - Bst clones?  
But where is vector  
background?



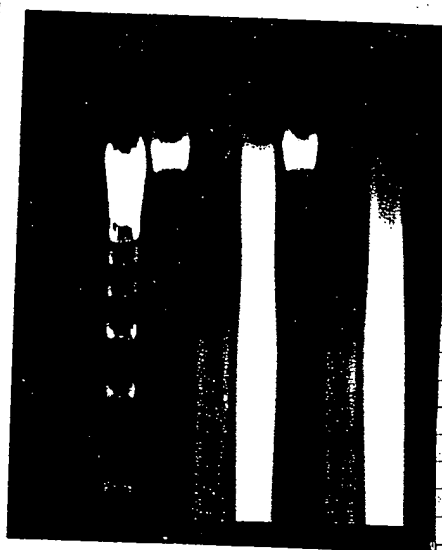
WDZ is made  
4/24/87



4/24

Restriction digestion  
of PCR products  
with Pst I  
(PCR primers 1, 2)

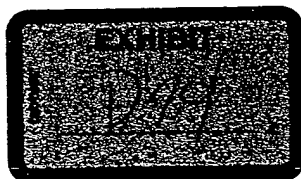
- 1) Ladder
- 2) pGEM
- 3) 101
- 4) 111
- 5) 101-1



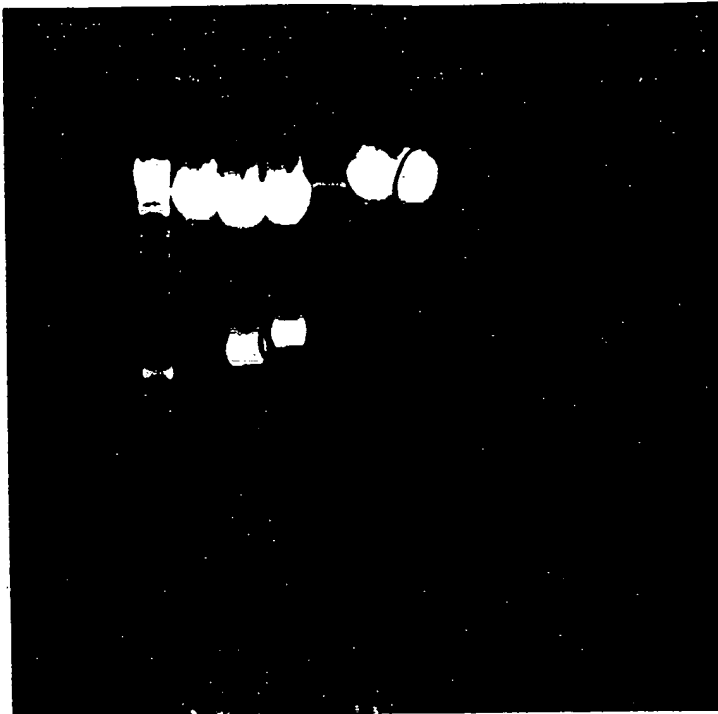
- 1) Ladder
- 2) 1101/1107 Pst I
- 3) 101-1 Pst I
- 4) 111-1 Pst I
- 5) 1101/1107 Bam HI
- 6) 101-1 Bam HI
- 7) 111-1 Xba I

Conclusion: 101-1 is rec.

111-1 ?



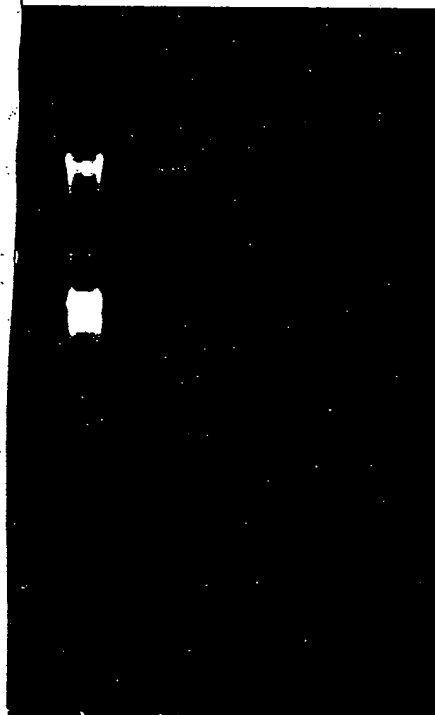
50 SHEETS  
22-141 100 SHEETS  
22-142 200 SHEETS  
22-144



4/26.

- 1) ladder
- 2) pCRIT/gp19uak7 (erry) / Kp1
- 3) ————— Kp1
- 4) ————— Bam + X6
- 5) pC1/gp19u (erry) / Eco R1
- 6) pCDNAS / Eco R1
- 7) pCDNAS / Bam + X6.

- 1) ladder
- 2) gp19uakids Bam-X6 fr.
- 3) pCDNAS Bam-X6 vector.

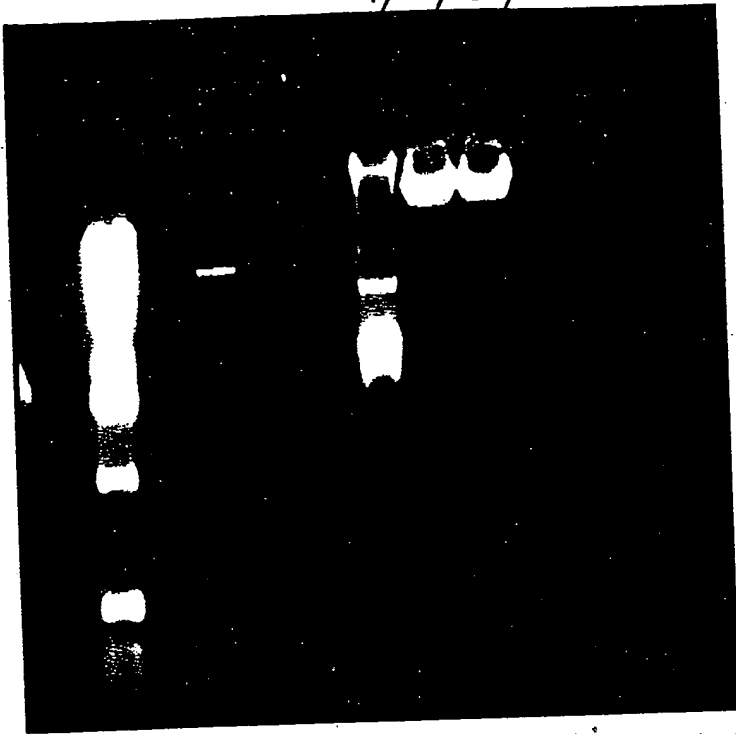


Ligation:

- 1) vector 5 $\mu$ l
- 2) vector lig 5 $\mu$ l
- 3) vector + insert  
5 $\mu$ l 5 $\mu$ l



1) 2/31



1) Coldder

2) PCR 18p19u AAS/Bent  
8511071  
3) - - - - - /A511071f  
X6e

If doesn't  
look like AAS 7p19u.

check with Pst1  
check with Terry!

4127

1) Coldder

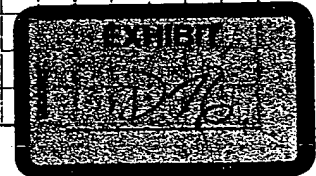
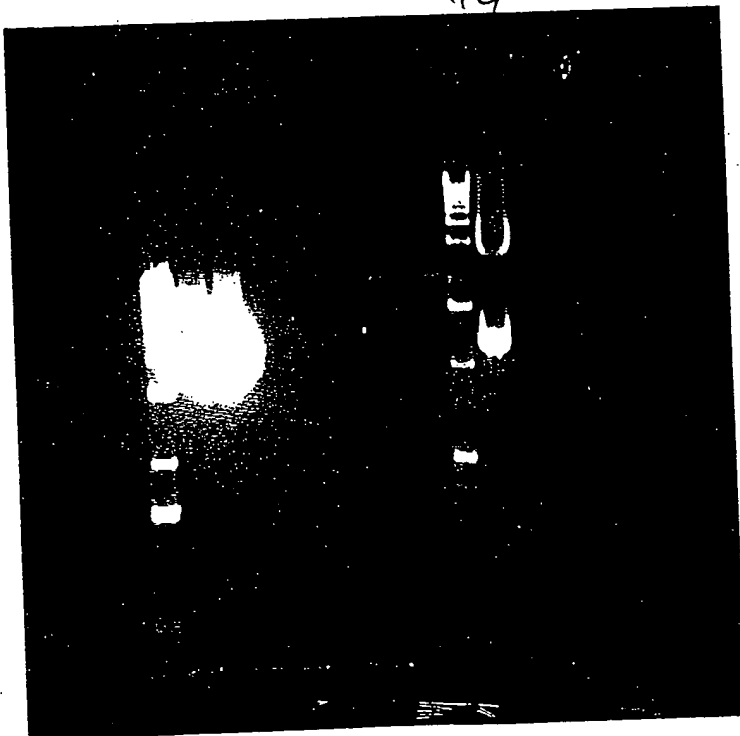
2) PCR 18p19u AAS/Pst

there is small  
fragment!

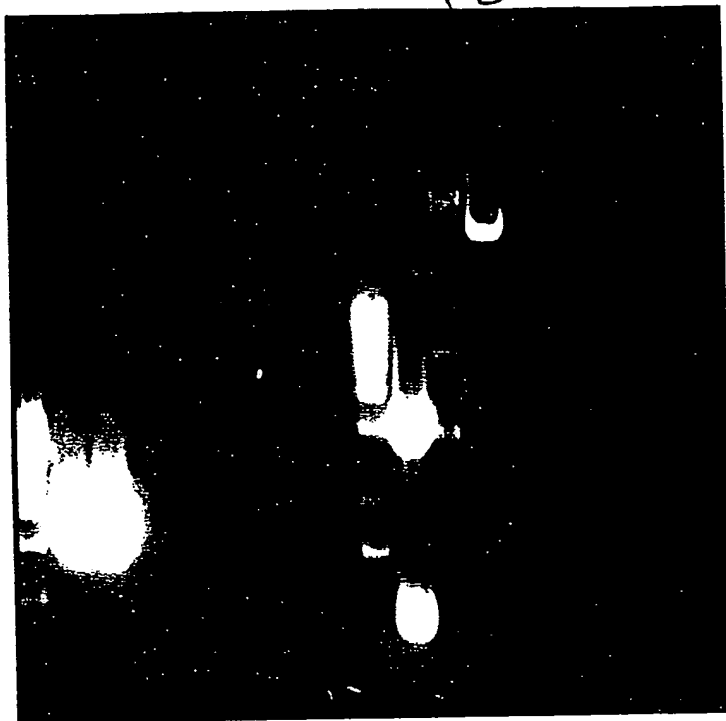
this is 412 8p 63 a  
in reverse orientation!?

check with Van TH

11124

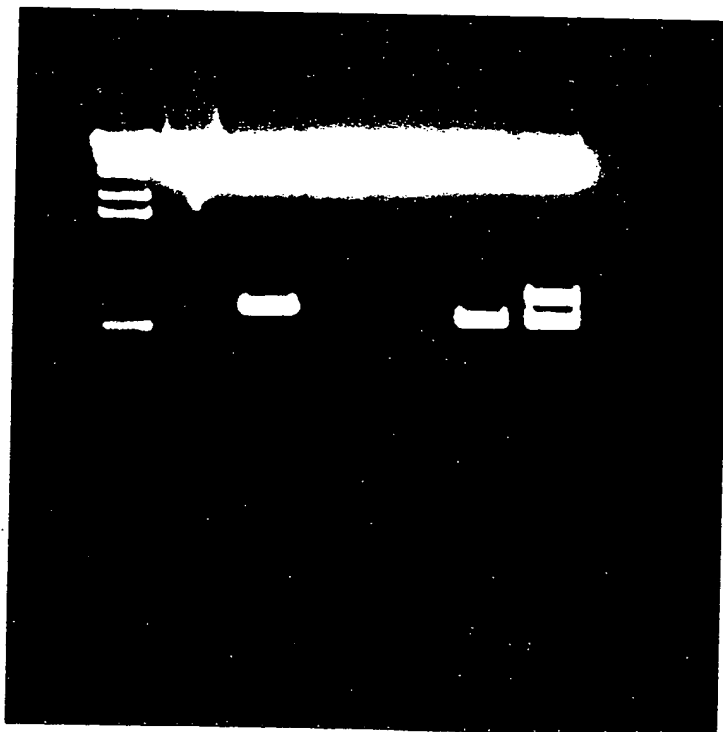


22-141 50 SHEETS  
22-142 100 SHEETS  
22-144 200 SHEETS



4/28.

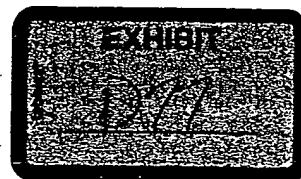
- 1) ladder.
- 1) pcr4 / 8p19k / HindIII
- ~ 500 bp fragment?



4/28.

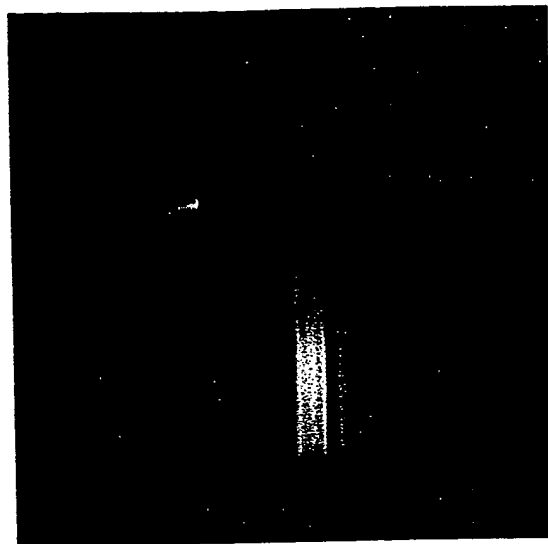
PCR / 8p19k  
restriction digestion

- 1) ladder
- 2) pcr1 / 8p19k
- 3) ——— / EcoRI
- 4) ——— / KpnI
- 5) ——— / PstI
- 6) ——— / HindIII
- 7) ——— / HindIII  
8000bp



4/30/37

4/24/97 Restriction digestion of 111 plagues



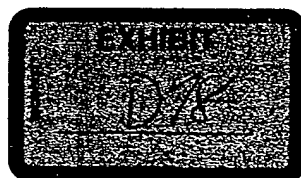
- 1) ladder
  - 2) 111/1107 DNA
  - 3) 111-1 (new prep) Bam K1f
  - 4) 111-2
  - 5) 111-3
  - 6) 111-4
- 111-2, 3, 4 - look like recombinants

4/30/97  
PCR analysis of  
111 plagues,  
Miners K91, K97



- 1) ladder
- 2) blank
- 3) pSV
- 4) pPG140a
- 5) p111
- 6) 111-1
- 7) 111-2
- 8) 111-3
- 9) 111-4

Conclusion is → all plagues are



22-141 50 SHEETS  
22-142 100 SHEETS  
22-144 200 SHEETS

4/30. Ligation:

cloning experiment 14. (14 ex).

- 1) Vector p223 / Bst 11071 + Xba I (PEB prep.)
- 2) Vector p223 / Bst 11071 + Xba I (Wikit prep.)
- 3) Insert 1101/1107 PCR ITR / Bst 11071 + Xba I  
(without gel purification).

S15. Comparing growth properties of: (plaqueing efficiency)

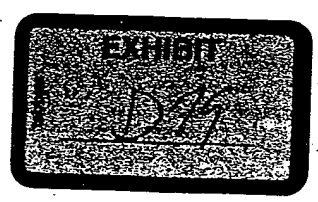
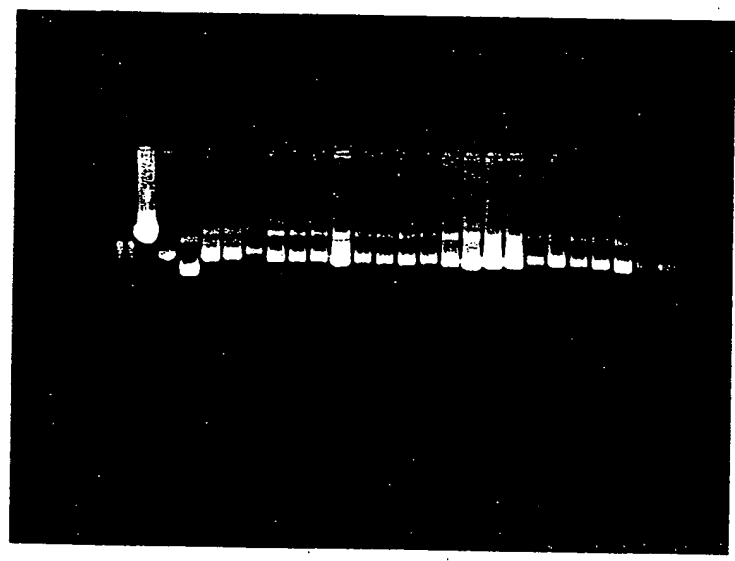
|          |          |                   |
|----------|----------|-------------------|
| 54 (4)   | Controls | 309               |
| 101 (-1) |          | 1101/1107         |
| 111 (-1) |          | 7001              |
| 111 (+2) |          | m1 m41 (pm 734.1) |

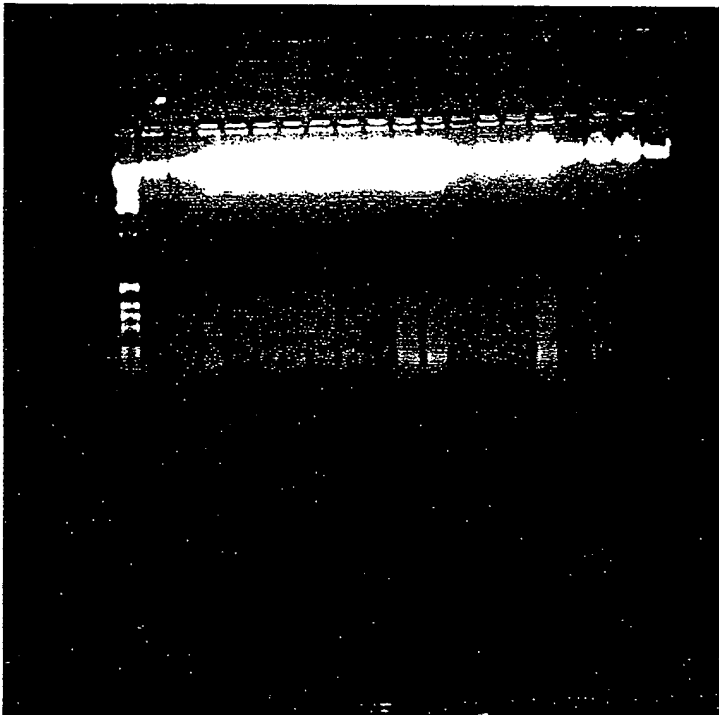
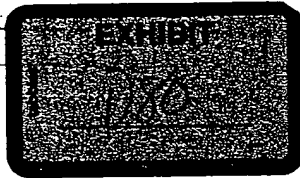
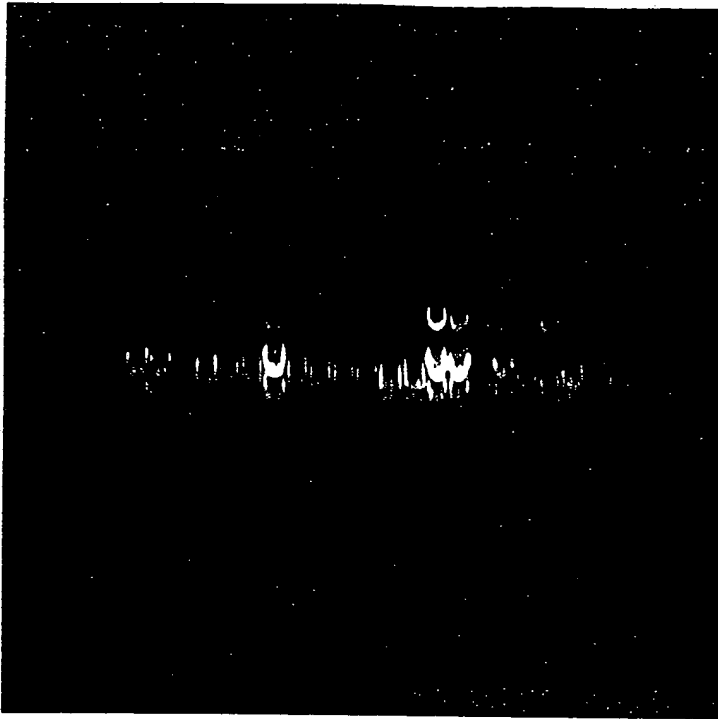
8/6/97

Minipreps of 14 ex.

- 1) ladder
- 2) p82
- 3) p223
- 4) 12ex-1
- 5)
- 6) 14ex1 (1-3)
- 7)
- 9) 14ex2 (1-20)
- 10)
- ↓
- 20)

⇒ 12ex-1 - junk.





4/6/97

1) Caddell

XQs + Ndc

↓ restriction of  
~~222~~, 14 ex clones

2) p223

141 (A3)

↓ 142 (1-20)

XQs +  
Ndc.

Caddell

141-3

142

-1, 2, 3,

5, 6, 7, 8, 9, 10,

13,

15, 16, 17, 18, 19

invariants

4/7/97

1) Caddell

2) p223

3) 141 (3)

4) 142 (1)

5) 142 (2)

6) 142 (3)

7) 142 (5)

8) 142 (6)

9) 142 (7)

10) 142 (8)

11) 142 (9)

12) 142 (10)

13) 142 (13)

14) 142 (15)

15) 142 (16)

16) 142 (17)

17) 142 (18)

18) 142 (19)

19) 141 (11) (wt)

All rec  
clones  
from A  
are  
rec-ts.

BS 217E

(BS) 1107E

+ XG 2

20/192/19  
wt)



4/8

# Sequencing

4) 25 - p141(3) - primer 4) 16  
 4) 26 - p141(3) - 4) 17  
 4) 27 - p142(1) - 4) 16  
 4) 28 - p142(1) - 4) 17

sequencing  
Didn't work

sequence is OK.

4/14. properly vectors pBSSK(+)/Sma I. ncl. 30°C  
p142(1) Bam + Nde. hc

rec 700, pm 734.1 predicted  
fragments length for SrfI-Nde  
digestion:

19549

7981

4846

3559

## Ligation:

Cloning exp 15 (p142(1) Bam + Nde + pEG140  
Bam-Nde Bfr.)

Cloning exp 16 (pBSSK Sma + rec700/Srf + Nde + T4pol)

Cloning exp 17 (pBSSK Sma + pm 734.1/Srf + Nde + T4pol)

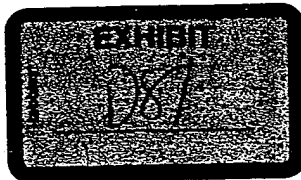
5/16 Results of transform. DMS α with 15, 16, 17 ex:

15V - 1  
 15VL - 14  
 15ex - 25

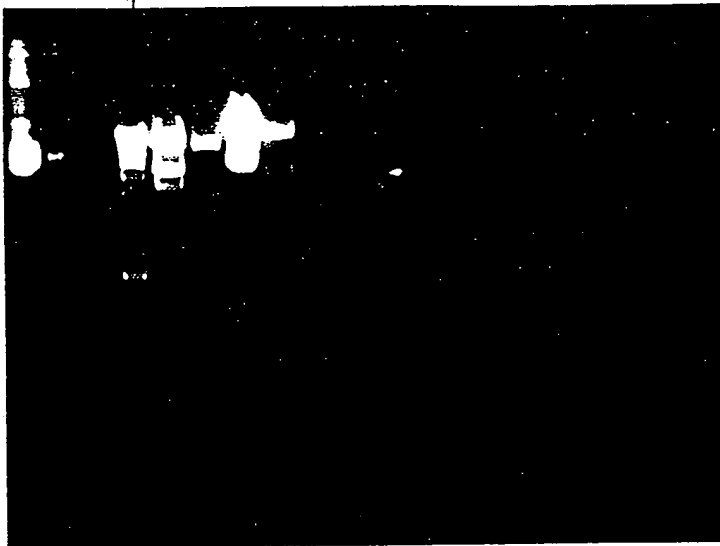
15-2 - MW 82  
 others either wt 142-1 or small  
 plasmids

16V - 0  
 16VL - 2R + 1W  
 16 ex - 5R + 19W  
 17 ex - 7W

} all wt or same MW



12 34



4/20

- 1) p82
- 2) p152

ladder run;

1) ladder

2) p82 } Barnett  
3) p152 } Ndel

4) p82 } Ndel

5) p152 } X631

Conclusion: 152 4's ok,  
making cell prep.

5/25 Manipulations of 17ex

17 ex - 2 (1 clear)  
- exercise fragment from get

- 1) ladder
- 2) p82E (+)
- 3) 17ex

↓

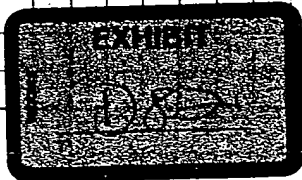
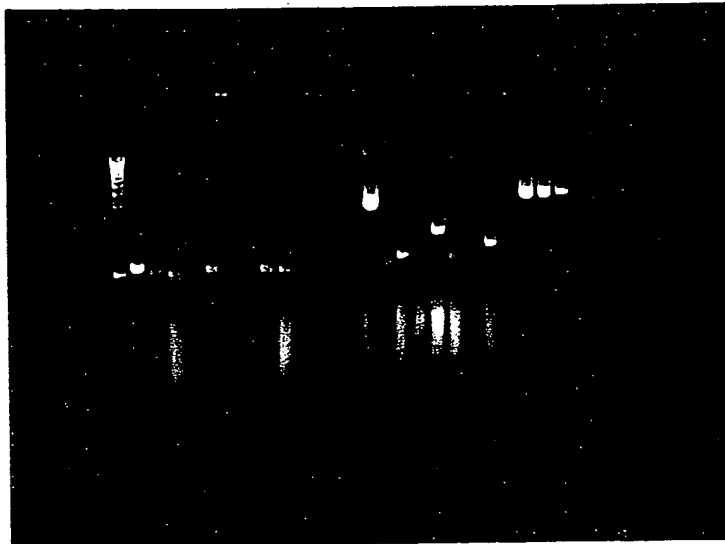
1-12

17ex2

1-12

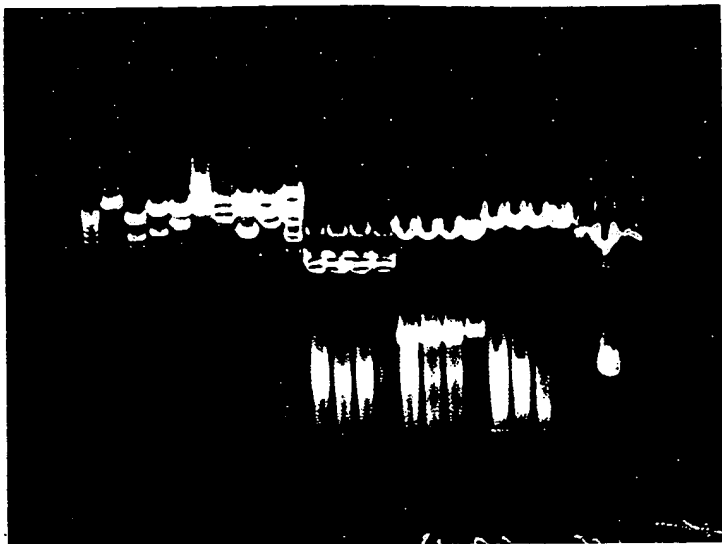
Take 172-1, 10, 11, 12

172-5 } possibly  
172-8 } some junk.



5/29/97

22-141 50 SHEETS  
22-142 100 SHEETS  
22-144 200 SHEETS



- 1) ladder
- 2) p82
- 3) p82 / Bam + Nde
- 4) p82 / Nde + Xba
- 5) p82 / bst 1107
- 6) p152
- 7) p152 / Bam + Nde
- 8) p152 / Nde + Xba
- 9) p152 / bst 1107
- 10) 7341 (Srf + Nde)
- 11) 1721
- 12) 17210
- 13) 17211
- 14) 17212
- 15) 1721
- 16) 17210
- 17) 17211
- 18) 17212
- 19) 1721
- 20) 17210
- 21) 17211
- 22) 17212
- 23) pCIGp191C / Xba + Xho
- 24) 1101/1107 / Spe + EcoRI

Conclusion: 152 is as expected.

17ex clones have correct insert in some (direct) orientation (EcoRI digestion). Xba doesn't cut insert due to methylation of Xba site.

The insert could be cut out with BamI-SalI to clone it into pCDNA3.1 BamI-XhoI sites.



5/28/97

Transfection 1101/1107 EcoRI + SpeI + p152.

6 dishes 293/TTC

8 dishes 293

293 TTC

2 dishes - control (1 ml)

2 - TTC (1 ml)

4 dishes - experiment 2 - + Ctrl/TTC (1 ml)

5/29/97

Cloning 18 ex:

172 BamI + SalI + XbaI (last)  $\Rightarrow$  3.5 kb (+)  
fragment (1.1 kb) + CIP  $\Rightarrow$  (buffer)

pCDNA3.1 BamI + XbaI  $\Rightarrow$  vector  
700  $\Rightarrow$  (buffer)  
50 kb

V(9M) - 8, VL  $\approx$  30, ex  $\sim$  300; LC(10M) - 0

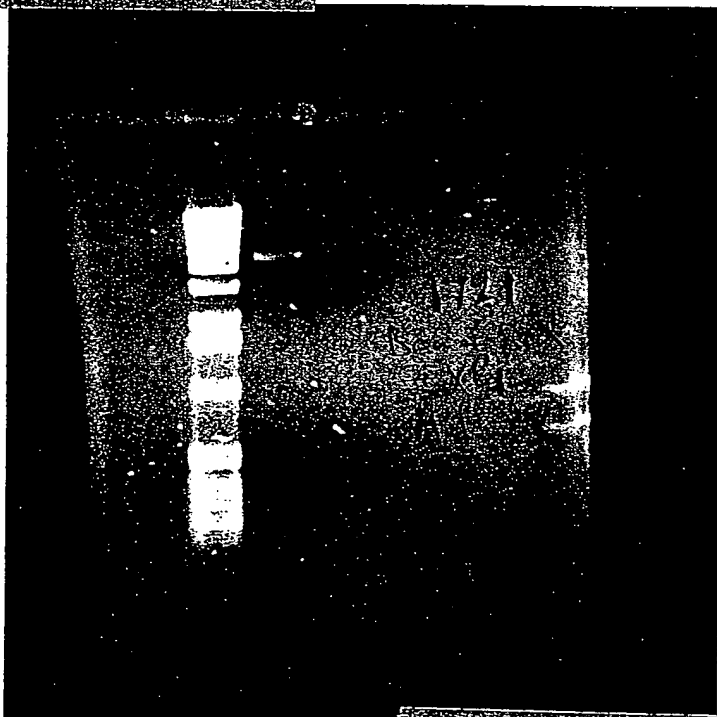


EXHIBIT  
D84

First cell culture experiment with 401, 2-3 5/15/97

5/15/97 Plaque assay of E3 viruses 293 cells

| Day      | Number of plaques | plaque size (mm) |           |          |          |        |              |              |              |     |
|----------|-------------------|------------------|-----------|----------|----------|--------|--------------|--------------|--------------|-----|
| 309      | (~2) 1/11         | 8/12             | ~100/1-5  | ~300/1-5 | ~500/1-5 | same   | complete CPE | ~1000        | complete CPE |     |
| (~1)     | 0/0               | 0/0              | ~20/1-2   | ~50/1-3  | ~100/1-5 | same   | ~100         | ~200         |              |     |
| 110/1107 | (~2) 0/0          | 0/0              | ~50/1-2   | ~30/1-5  | ~500/1-5 | same   | ~100         | ~500         |              |     |
| (~1)     | 0/0               | 0/0              | 5/1       | 10/1-2   | 10/1-2   | same   | ~30          | 50           |              |     |
| 7001     | (~3) 0/0          | 0/0              | 0/0       | 0/0      | 0/0      | 0/0    | 0/0          | 0/0          | 0/0          |     |
| (~2)     | 0/0               | 0/0              | 0/0       | 0/0      | 0/0      | 0/0    | 0/0          | 0/0          | 0/0          |     |
| pm T34.1 | (~4) 0/0          | 0/0              | 0/0       | 0/0      | 0/0      | 0/0    | 0/0          | 0/0          | 0/0          | ~30 |
| (~3)     | 0/0               | 0/0              | 0/0       | 0/0      | 0/0      | 0/0    | 0/0          | 0/0          | 0/0          | 0   |
| (~2)     | 0/0               | 0/0              | 0/0       | 0/0      | 0/0      | 0/0    | 0/0          | 0/0          | 0/0          | 0   |
| 544      | (~4) 0/0          | 0/0              | 0/1       | 8/1-2    | 20/1-2   | 30/1-2 | ~60/1-2      | ~100         | ~100         |     |
| (~5)     | 0/0               | 0/0              | 0/0       | 1/1      | 4/1-2    | same   | 0/1-1        | 30           |              |     |
| 101-1    | (~4) 0/1          | 1/1-2            | >100/1-5  | >300/1-5 | >1000    | same   | complete CPE | Complete CPE |              |     |
| (~3)     | 0/0               | 0/0              | ~50/1-5   | ~80/1-5  | >100     | same   | >300         | >300         |              |     |
| (~2)     | 0/0               | 0/1              | ~15/1-5   | ~30/1-5  | >30      | same   | >20          | 200          | 100          |     |
| 111-1    | (~4) 0/0          | 0/2-3            | >100/1-10 | >300/1-5 | >1000    | same   | complete CPE | complete CPE |              |     |
| (~3)     | 0/0               | 1/8              | ~50/1-10  | ~80/1-5  | ~80      | same   | ~300         | >300         |              |     |
| (~2)     | 0/0               | 0/0              | 10/1      | 30/1-3   | ~50      | same   | ~50          | 200          | >50          |     |
| 111-2    | (~4) 0/0          | 0/1-5            | 20/1-2    | 50/1-5   | 90       | same   | ~100         | >100         |              |     |
| (~3)     | 0/0               | 1/1              | 10/1      | 20/1-5   | 90       | same   | ~50          | >50          |              |     |
| (~2)     | 0/0               | 0/0              | 0/0       | 0/0      | 10       | same   | 2/3          | 2/8          |              |     |
| 309      | (~2) CPE          | CPE              | CPE       | CPE      | CPE      | CPE    | CPE          | CPE          | CPE          |     |
| 110/1107 | (~2) CPE          | CPE              | CPE       | CPE      | CPE      | CPE    | CPE          | CPE          | CPE          |     |
| 7001     | (~3) 2            | 3                | 3         | 3        | 3        |        |              |              |              |     |
| (~1)     | 0                 | 1                | 3         | 3        | 5        |        |              |              |              |     |
| pm T34.1 | (~4) ~50          | ~60              | ~60       | ~70      | ~70      |        |              |              |              |     |
| (~3)     | 4                 | 7                | 9         | 10       | 16       | 2/3    | 30           |              |              |     |
| (~2)     | 0                 | 0                | 1         | 1        | 2        |        |              |              |              |     |
| 544      | (~4) ~100         | ~100             | ~100      | ~100     |          |        |              |              |              |     |
| (~3)     | 33                | 33               | 33        | 33       |          |        |              |              |              |     |
| 101-1    | (~4) CPE          | CPE              | CPE       | CPE      |          |        |              |              |              |     |
| (~3)     | CPE               | CPE              | CPE       | CPE      |          |        |              |              |              |     |
| (~2)     | CPE               | CPE              | CPE       | CPE      |          |        |              |              |              |     |
| 111-1    | (~4) CPE          | CPE              | CPE       | CPE      |          |        |              |              |              |     |
| (~3)     | CPE               | CPE              | CPE       | CPE      |          |        |              |              |              |     |
| (~2)     | 50                | 50               |           |          |          |        |              |              |              |     |
| 111-2    | (~4) >600         | ~100             |           |          |          |        |              |              |              |     |
| (~3)     | >50               | ~50              |           |          |          |        |              |              |              |     |



715 100 APT in CS program - development on 1/5/44.

deg = 5 6 7 8 Friday Saturday

(12+1) (14) (15) (18)

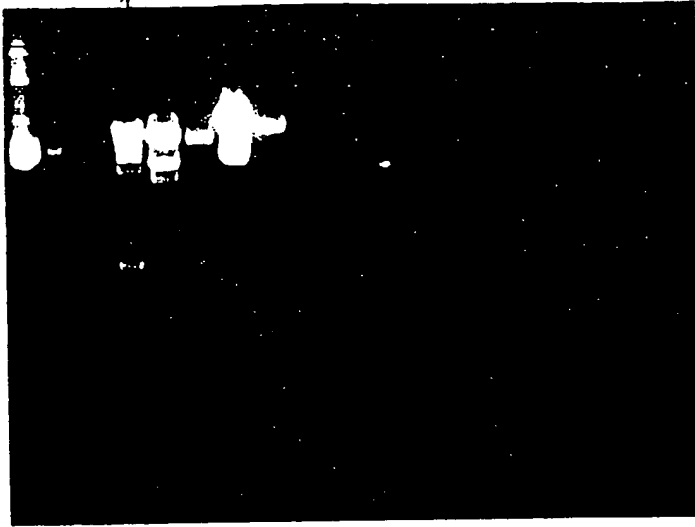
|              | 12 | 52 | ~100               | ~100 | ~100 | CCPE | CCPE |    |    |
|--------------|----|----|--------------------|------|------|------|------|----|----|
| 309 (~1)     | 12 | 52 | ~100               | ~100 | ~100 | CCPE | CCPE |    |    |
| (~0.1-1)     | 1  | 5  | 14                 | 19   | 20   |      |      |    |    |
| (~0.1-2)     | 3  | 7  | 19                 | 26   | 27   |      |      |    |    |
| 104/107 (~1) | 0  | 0  | 3 <sub>small</sub> | 8    | 15   |      |      |    |    |
| (~0.1-1)     | 0  | 0  | 1                  | 3    | 3    | 5    | 6    |    |    |
| (~0.1-2)     | 0  | 0  | 0                  | 0    | 1    | 2    | 2    |    |    |
| 7001 (~3)    | 0  | 0  | 0                  | 0    | 0    | 0    | 0    | 2  | 2  |
| (~2)         | 0  | 0  | 0                  | 0    | 0    | 0    | 0    |    |    |
| 734.1 (~3)   | 0  | 0  | 0                  | 0    | 0    | 8    | 8    | 11 | 15 |
| (~2)         | 0  | 0  | 0                  | 0    | 0    | 4    | 5    | 6  | 9  |
| 101-1 (~3)   | 1  | 11 | 48                 | 60   | ~60  |      |      |    |    |
| (~2)         | 3? | 7  | 20                 | 30   | 54   |      |      |    |    |
| 01-1 (~1)    | 0  | 2  | 3                  | 4    | 7    | 9    |      | 11 |    |
| 11-1 (~3)    | 0  | 6  | 35                 | 50   | ~50  |      |      |    |    |
| (~2)         | 0  | 0  | 5                  | 9    | 16   |      |      |    |    |
| (~1)         | 0  | 0  | 2                  | 4    | 4    |      |      | 8  | 11 |
| 11-2 (~4)    | 3? | 30 | 70                 | 100  | ~100 |      |      |    |    |
| (~3)         | 2? | 4  | 16                 | 20   | 30   |      |      |    |    |
| (~2)         | 0  | 0  | 1                  | 4    | 4    | 6    |      |    |    |
| (~1)         | 0  | 0  | 0                  | 0    | 0    | 0    |      | 7  |    |

EXHIBIT  
1 D86



22-141 50 SHEETS  
22-142 100 SHEETS  
22-144 200 SHEETS

12 3 4



|   |   |   |   |
|---|---|---|---|
| 4 | 1 | 2 | 0 |
|---|---|---|---|

11-18-2

27 p 152

10/11/1944

7. Calder

2) p 82

Bonus

Nde!

11/21/24

763

Conchiser 1 152 45 min,  
making cell prep.

5/25 Whirpools of 17 ex

Ex - 2 (Review)

exercice fréquent pour gel

1. ladder

$$2) \text{ pbsk}(t)$$

5. 17x

|   |   |   |
|---|---|---|
| 1 | 2 | 3 |
|---|---|---|

17-08-2

|   |   |   |
|---|---|---|
| 1 | 1 | 1 |
|---|---|---|

Take  $72-1, 10, 11, 12$

172-5

172-8

pass. 42

1. Soup Sink.

## EXHIBIT

D87

8944, 5/5/97, 5/23/97

Ray (Plagues)

Urus: 5 6 7 8 9 10 11 12 13 14 15 16 17 18

300

4 12 33 45 47  
8.5 25 70 95 100

#

1101/1107

18 14 18  
19 19 185

207 21  
95 100

7001

734.1

12 13 11 24  
50 54 70 100

544

14 25 91 42  
133 53 97 100

101-1

3 9 23 34 41 43  
7 20 53 79 95 100

111-1

2 4 16 29 39  
7 13 53 66 100

EXHIBIT

D88



50 SHEETS  
22-141 100 SHEETS  
22-142 200 SHEETS  
22-144



5/15/97 Plaque development on 295 (1. plaques)

mins <sup>day</sup> 4 5 6 7 8 10 14 15 16 18 19 22 23 24  
309 10 25 50 100 100

110/1107 8.3 16 50 83 100

700/ ~~25 50 75 100~~ ?

734.1 ~~(133 233 30 33 53 76 100)~~ !

544. 3 12 27 90 100

101-1. (1) 15 30 70 100

111-1 ~~20 60 100~~ ?

111-2 21 36 69 84 96 100

5/15/97 ↑ Same, 1549.

5 6 7 8 9 12 13 14  
309 11 25 70 96 100

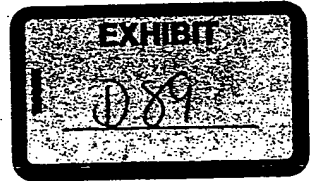
110/1107 16 50 83 100

~~734.1~~ TO 76 100 ?

101-1 18 27 36 63 82 100

111-1 25 50 100

111-2 16 66 100



# 5/19/97 Plaque assay of ES viruses 293 cells

| Day       | Number of plaques | Plaque size (mm) | ADD       |
|-----------|-------------------|------------------|-----------|
| 309       | (~2) 1/1          | 8/12             | ~100/1-5  |
| (~1)      | 0/0               | 0/0              | ~20/1-2   |
| 1107      | (~2) 0/0          | 0/0              | ~50/1-2   |
| (~1)      | 0/0               | 0/0              | 5/1       |
| 115       | (~3) 0/0          | 0/0              | 0/0       |
| (~2)      | 0/0               | 0/0              | 0/0       |
| T34.1     | (~4) 0/0          | 0/0              | 0/0       |
| (~3)      | 0/0               | 0/0              | 0/0       |
| (~2)      | 0/0               | 0/0              | 0/0       |
| 14        | (~4) 0/0          | 0/0              | 10/1      |
| (~3)      | 0/0               | 0/0              | 0/0       |
| -1        | (~4) 6/1          | 13/1-2           | >100/1-5  |
| (~3)      | 0/0               | 0/0              | ~50/1-5   |
| (~2)      | 0/0               | 0/1              | ~15/1-5   |
| 11-1      | (~4) 6/1          | 9/2-8            | >100/1-10 |
| (~3)      | 0/0               | 1/8              | ~50/1-10  |
| (~2)      | 0/0               | 0/0              | 10/1      |
| 111-2     | (~4) 0/0          | 8/1-5            | 20/1-2    |
| (~3)      | 0/0               | 1/1              | 10/1      |
| (~2)      | 0/0               | 0/0              | 0/0       |
| 309       | (~2) CCPE         | CCPE             | CCPE      |
| (~1) CCPE | CCPE              | CCPE             | CCPE      |
| 1107      | (~2) 25           | 50               | 60        |
| 7001      | (~3) 2            | 3                | 3         |
| (~2) 0    | 1                 | 3                | 3         |
| T34.1     | (~4) ~50          | ~60              | ~70       |
| (~3) 4    | 7                 | 9                | 10        |
| (~2) 0    | 0                 | 1                | 2         |
| 544       | (~4) ~100         | ~100             | ~100      |
| (~3) 33   | 33                | 33               | 33        |
| 101-1     | (~4) CCPE         | CCPE             | CCPE      |
| (~3) CCPE | CCPE              | CCPE             | CCPE      |
| (~2) CCPE | CCPE              | CCPE             | CCPE      |
| 111-1     | (~4) CCPE         | CCPE             | CCPE      |
| (~3) CCPE | CCPE              | CCPE             | CCPE      |
| (~2) ~50  | 50                | 50               | 50        |
| 111-2     | (~4) >100         | ~100             | ~100      |
| (~3) >50  | ~50               | ~50              | ~50       |
| (~2) 32   | 33                | 33               | 33        |

First cell culture experiment with K01-2-3 5/15/97

First cell culture experiment with  
K01-2-3  
5/15/97



5/15 Rec ADP & ES plaque development on ASU4

day = 5 6 7 8 Friday Saturday

(12+1) (14) (15) (16)

CCPE

|              |    |    |       |      |      |      |   |    |    |
|--------------|----|----|-------|------|------|------|---|----|----|
| 809 (~1)     | 12 | 52 | ~100  | ~100 | ~100 | CCPE |   |    |    |
| (~0.1+1)     | 1  | 5  | 14    | 19   | 20   |      |   |    |    |
| (~0.1-2)     | 3  | 7  | 19    | 26   | 27   |      |   |    |    |
| 101/102 (~1) | 0  | 0  | 3 smd | 8    | 15   |      |   |    |    |
| (~0.1+1)     | 0  | 0  | 1     | 3    | 3    | 5    | 6 |    |    |
| (~0.1-2)     | 0  | 0  | 0     | 0    | 1    | 2    | 2 |    |    |
| 7001 (~3)    | 0  | 0  | 0     | 0    | 0    | 0    | 0 | 2  | 2  |
| (~2)         | 0  | 0  | 0     | 0    | 0    | 0    | 0 |    |    |
| 734.1 (~3)   | 0  | 0  | 0     | 0    | 0    | 8    | 8 | 11 | 15 |
| (~2)         | 0  | 0  | 0     | 0    | 0    | 4    | 5 | 6  | 9  |
| 101-1 (~3)   | 1  | 4  | 48    | 60   | ~60  |      |   |    |    |
| (~2)         | 3  | 7  | 20    | 30   | 54   |      |   |    |    |
| 01-1 (~1)    | 0  | 2  | 3     | 4    | 7    | 9    |   |    |    |
| 11-1 (~3)    | 0  | 6  | 35    | 50   | ~50  |      |   |    |    |
| (~2)         | 0  | 0  | 5     | 9    | 16   |      |   |    |    |
| (~1)         | 0  | 0  | 2     | 4    | 4    |      |   | 8  | 11 |
| 11-2 (~4)    | 3  | 30 | 70    | 100  | ~100 |      |   |    |    |
| (~3)         | 2  | 4  | 16    | 20   | 30   |      |   |    |    |
| (~2)         | 0  | 0  | 1     | 4    | 4    | 6    |   |    | 7  |
| (~1)         | 0  | 0  | 0     | 0    | 0    | 0    |   |    |    |

EXHIBIT  
1 D91

22-141 50 SHEETS  
22-142 100 SHEETS  
22-146 200 SHEETS

5/21/97 Playm essay of E3 viruses in ASU,  
(manalaya) is dying, data are not quantitative

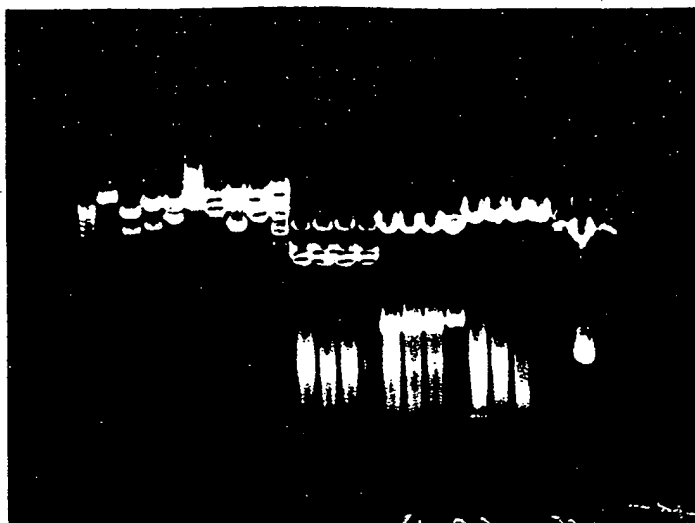
22-141 50 SHEETS  
22-142 100 SHEETS  
22-143 200 SHEETS



| Time | Day, ploques                | 6            | 7             | 8               | 12          | 14 |
|------|-----------------------------|--------------|---------------|-----------------|-------------|----|
| 309  | (~2) ~30<br>(~1) 3          | 12           | 33            |                 |             |    |
| 1107 | (~3) 0<br>(~2) 0<br>(~1) 0  | 3<br>3<br>0  | 17<br>17<br>6 | 2.7<br>3.0<br>7 |             |    |
| 1100 | (~4) 0<br>(~3) 0            | 0<br>0       | 1<br>0        | 1<br>0          | 1<br>0      |    |
| 734  | (~4) 0<br>(~3) 0            | 0<br>0       | 0<br>0        | 2<br>3          | 3           |    |
| 544  | (~4) 14<br>(~3) 0<br>(~2) 0 | 24<br>0<br>0 | 239<br>2<br>0 | 3<br>0<br>0     | 3<br>0<br>0 |    |
| 1011 | (~3) 0<br>(~2) 0            | 11<br>3      | 28<br>11      | 12<br>5         |             |    |
| 1111 | (~3) 0<br>(~2) 0            | 0<br>0       | 3<br>7        | 14              |             |    |
| 1112 | (~3) 0<br>(~2) 0<br>(~1) 0  | 7<br>0<br>0  | 27<br>1       | 2<br>0          |             |    |

EXHIBIT

D92



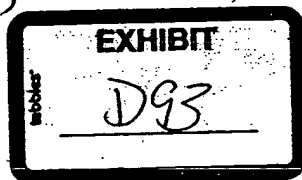
5/29/97

- 1) p82
- 2) p82
- 3) p82 / Bam + Nde
- 4) p82 / Nde + Xba
- 5) p82 / Bst 1107
- 6) p152
- 7) p152 / Bam + Nde
- 8) p152 / Nde + Xba
- 9) p152 / Bst 1107
- 10) 7341 / Srf + Nde
- 11) 1721
- 12) 17210
- 13) 17211
- 14) 17212
- 15) 1721
- 16) 17210
- 17) 17211
- 18) 17212
- 19) 1721
- 20) 17210
- 21) 17211
- 22) 17212
- 23) pC1gp191C / Xba + Xho
- 24) 1101/1107 / Spe + EcoRI

Conclusion: 152 is as expected.

17ex clones have correct insert in some (direct) orientation (EcoRI digestion). Xba doesn't cut insert due to methylation of Xba site.

The insert could be cut out with BamI-SbfI to clone it into pCDNA3.1 BamI-XhoI sites.



5/28/97

Transfection 1101/1107 EcoRI + SpeI + p152.

6 dishes 293/TTC  
8 dishes 293

293 TTC

2 dishes - control (1 ml)  
4 dishes - experiment 2 - TTC (1 ml)  
2 - + env/TTC (1 ml)

5/29/97

Cloning 18 ex:

172 BamI + SalI + XbaI (EcoRI)  $\Rightarrow$  3.5 kb (A)  
(fragment (1' cut) + CIP  $\Rightarrow$  (buffer))

pCDNA3 BamI + XbaI  $\Rightarrow$  vector  
700 bp  $\Rightarrow$  (buffer)

V(9M) - 0, VL  $\approx$  30, ex  $\approx$  300, L (10  $\mu$ l) - 0

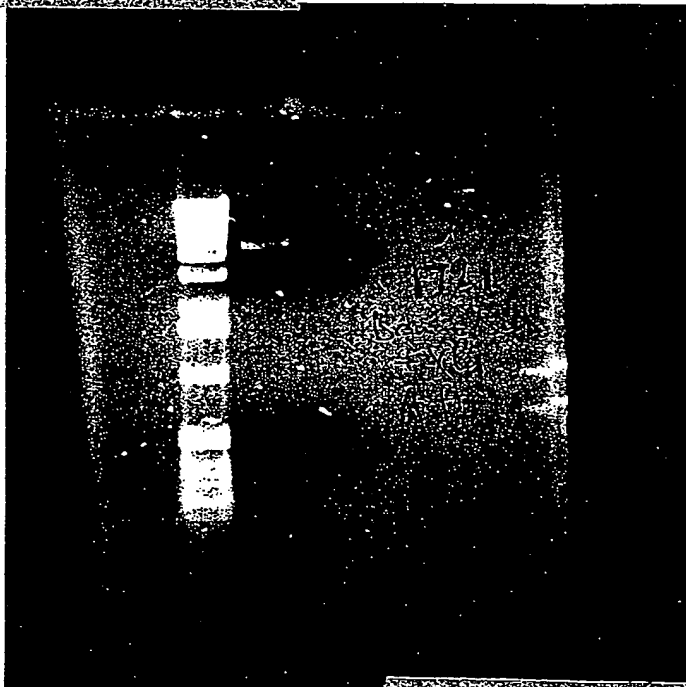


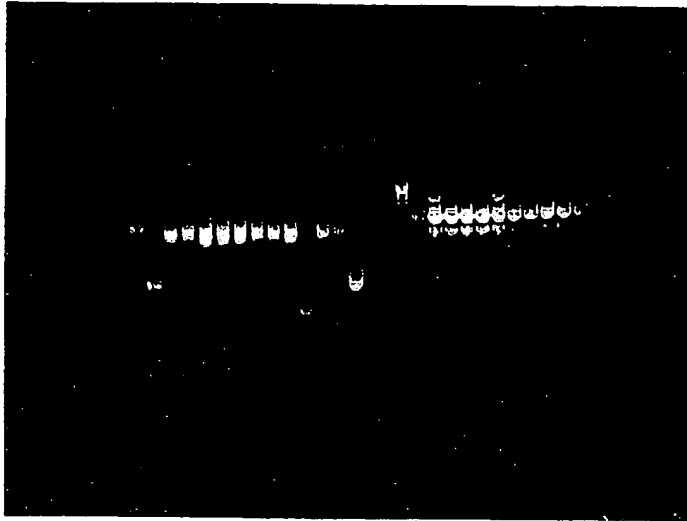
EXHIBIT  
D96

6/5/97

18 ex miniprep

⇒ 1-8, 10, 11 - look like  
rectis  
great  
(~~great~~ NW)

22-141 50 SHEETS  
22-142 100 SHEETS  
22-144 200 SHEETS



1) Ladder

2) ↓ 18 ex (1-12)

Take 1-8, 10, 11.

1) Ladder

2) pCDNA3.1Zeo(+)

3) ↓ 18 ex  
↓ 1-8, 10, 11

Bam  
Xba

Look like rect's.

Grow 18(1).

EXHIBIT

D95

6/5/47

VIPA - infections:

1101/1107, 544, 101-1, 111-1, 734.1

E - early (9-15)  
L - late (24-28)

| (295)E        | (293-1)E | (A549)E | (295) | (293)TC | (A549) |
|---------------|----------|---------|-------|---------|--------|
| M - 1         |          |         |       |         |        |
| 1101/1107 - 2 | 7        | 13      | 13    | 13      | 36     |
| 544 - 3       | 8        | 14      | 26    | 26      | 56     |
| 101-1 - 4     | 9        | 15      | 21    | 27      | 53     |
| 111-1 - 5     | 10       | 16      | 22    | 28      | 54     |
| 734.1 - 6     | 11       | 17      | 23    | 29      | 55     |
|               | 12       | 18      | 24    | 36      | 56     |

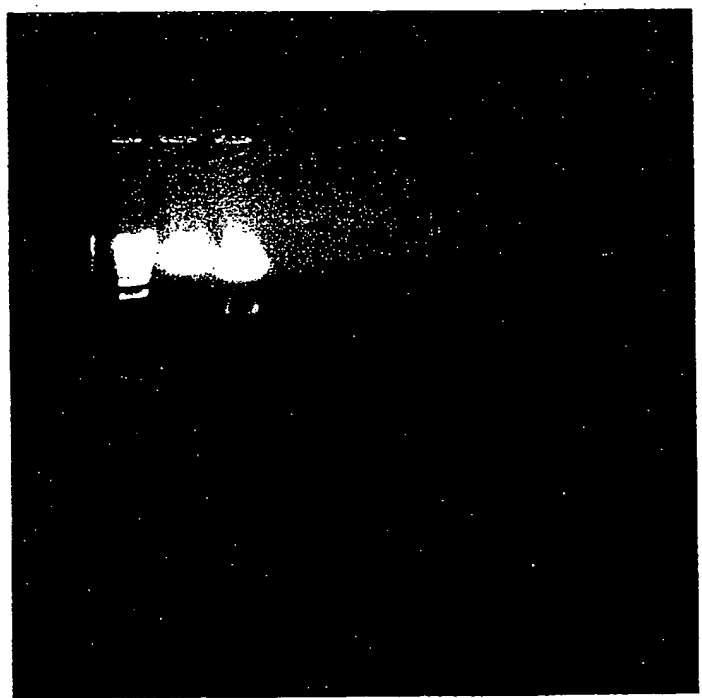
544

1101/1107 - 100μl  
544 - 100μl  
101 - 1 - 100μl  
111 - 2 - 150μl  
734.1 - 150μl

293

1101/1107 - 100μl  
544 - 100μl  
101 - 1 - 100μl  
111 - 2 - 500μl  
734.1 - 100μl

- Viruses dosage  
(and stock of viruses)



6/10/47  
Restriction pattern  
for 181 maxiprep.

- 1) Cadder
- 2) 181/Spel + PseI
- 3) 181/Spel + XhoI

Pattern is as expected.

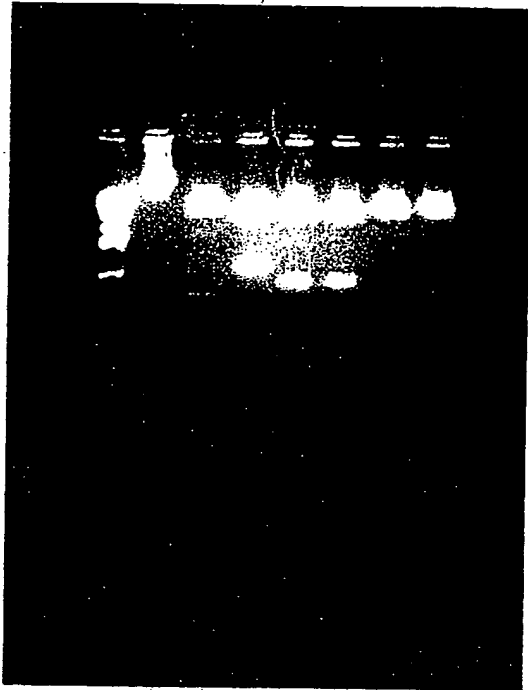
EXHIBIT  
D96



6/25/97

Restriction digestion of pCI-14.7 (Terry).

22-141 50 SHEETS  
22-142 100 SHEETS  
22-144 200 SHEETS



1) ladder

2) p14.7

3) — EcoRI

4) — BglII

5) — SpeI + XhoI

6) — SpeI + Salt

7) — BamHI + XhoI

8) — BamHI + Salt

7/1/97 Clonings 19 ex; 20 ex;

19 ex — vector p430 (SmaBI); insert 19 ex1 { p181  
mfe  
XbaI  
Klenow

19 ex2 { p181  
mfe  
RV  
Klenow

20 ex — vector pL2/PacI + T4; insert

20 ex1 p181  
MX KL

7/3 transformation MFL

20 V ~ 10

20 VL ~ 50

20 ex1 ~ 100

20 ex11 ~ 30

13 V ~ 20

19 VL ~ 100

19 ex1 ~ 100

19 ex11 ~ 100

20 ex2 p181

M RV KL

EXHIBIT

D97

## **EXHIBIT E**



Dear Dan:

Attached is a list of primers that were ordered by Konstantin Doronin. The primers were named KD1, KD2, KD3, KD4, KD5, etc. (see Description/Sequences). These primers were used in studies on the ADP-overexpressing vectors, either for DNA sequencing or for amplification of certain sequences by the polymerase chain reaction (PCR). Also attached are Dr. Doronin's handwritten notes indicating the use of the primers. (These notes are not dated, but this list was begun when the primers were ordered). Please note that the primers were ordered on 9/26/96, 11/04/96, and 03/24/97.

Also included are some actual DNA sequence data using some of these primers to sequence some of the plasmids that were used to construct the ADP-overexpressing vectors. The dates of the sequencing runs are on the datasheets. Note that the first sequencing run was on November 19, 1996. Other runs were on November 25/26, 1996, March 20/21, 1997, April 21, 1997, May 12, 1997, and March 23/24, 1999.

Thanks,

Bill

|   |            |          |
|---|------------|----------|
| Order Number                              |            |          |
| Please refer to this No. on all inquiries | Order Date | Page No. |
| 473935 A                                  | 09/26/98   | 1 of 1   |

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DORONIN  
000127752

|     |         |                     |
|-----|---------|---------------------|
| MLR | 5903851 | FAX/DEBBIE HUMISTON |
|-----|---------|---------------------|

|     |           |                                     |        |         |      |
|-----|-----------|-------------------------------------|--------|---------|------|
| 1.0 | 10336-022 | KD1 \ DORONIN                       | 1 Each | ( G07 ) | 19.3 |
|     | A3884G07  | CTACGAGAGAACCTCTCCGAG               |        |         |      |
| 2.0 | 10336-022 | KD2 \ DORONIN                       | 1 Each | ( G08 ) | 15.5 |
|     | A3884G08  | GCCACAACCTTATACTGTTTGC              |        |         |      |
| 3.0 | 10336-022 | KD3 \ DORONIN                       | 1 Each | ( G09 ) | 28.6 |
|     | A3884G09  | TCAGCCACGGTACTTAATTAACCCAAAAGGTGGAT |        |         |      |
| 4.0 | 10336-022 | KD4 \ DORONIN                       | 1 Each | ( G10 ) | 18.8 |
|     | A3884G10  | GCTCTAGAAGTCAGGCTTCCTGG             |        |         |      |
| 5.0 | 10336-022 | KD5 \ DORONIN                       | 1 Each | ( G11 ) | 17.8 |
|     | A3884G11  | GCTCTAGATCTCATTTAATCATA             |        |         |      |
| 6.0 | 10336-022 | KD6 \ DORONIN                       | 1 Each | ( G12 ) | 21.5 |
|     | A3884G12  | CCTTAATTAAGTCAGGCTTCCTGG            |        |         |      |
| 7.0 | 10336-022 | KD7 \ DORONIN                       | 1 Each | ( H01 ) | 20.3 |
|     | A3884H01  | CCTTAATTAATCTCATTTAATCATA           |        |         |      |
| 8.0 | 10336-022 | KD8 \ DORONIN                       | 1 Each | ( H02 ) | 21.7 |
|     | A3884H02  | CGCCTATACAGAAGATTTTCCAG             |        |         |      |
| 9.0 | 10336-022 | KD9 \ DORONIN                       | 1 Each | ( H03 ) | 20.5 |
|     | A3884H03  | CGCCTATACACTGCAGCAGGTGTG            |        |         |      |



**GIBCO BRL Custom Primers**  
*Certificate of Analysis*

|                         |
|-------------------------|
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| Order Number: 473935A   |
| Order Date: 09/26/96    |

**Primer 1:**

Primer Name: KD1  
Researcher: DORONIN  
Sequence (5' to 3'): CTA CGA GAG AAC CTC TCC GAG  
Molecular Weight  $\mu\text{g}/\mu\text{mole}$ : 6740.2  
Millimolar Extinction Coefficient: 230.5  
**Purity** **Deprotected**  
**Tm (1 M Na+)** **73**  
**Tm (50 mM Na+)** **51**  
**% GC** **57**

Primer Number: A3884G07 (G07)  
Primer Length: 21  
 $\mu\text{g}$  per OD: 29.2  
nmoles per OD: 4.3  
OD's 19.3  
 $\mu\text{g's}^*$  566  
nmoles 84  
Coupling Eff. 99%

Notes:



**Primer 2:**

Primer Name: KD2  
Researcher: DORONIN  
Sequence (5' to 3'): GCC ACA ACT TAT ACT GTT TGC  
Molecular Weight  $\mu\text{g}/\mu\text{mole}$ : 6696.2  
Millimolar Extinction Coefficient: 221.4  
**Purity** **Deprotected**  
**Tm (1 M Na+)** **67**  
**Tm (50 mM Na+)** **45**  
**% GC** **42**

Primer Number: A3884G08 (G08)  
Primer Length: 21  
 $\mu\text{g}$  per OD: 30.2  
nmoles per OD: 4.5  
OD's 15.5  
 $\mu\text{g's}^*$  471  
nmoles 70  
Coupling Eff. 99%

Notes:

**Primer 3:**

Primer Name: KD3  
Researcher: DORONIN  
Sequence (5' to 3'): TCA GCC CAC GGT ACT TAA TTA ACC CAA AAG GTG  
GAT  
Molecular Weight  $\mu\text{g}/\mu\text{mole}$ : 11632.2  
Millimolar Extinction Coefficient: 407.2  
**Purity** **Deprotected**  
**Tm (1 M Na+)** **81**  
**Tm (50 mM Na+)** **59**  
**% GC** **44**

Primer Number: A3884G09 (G09)  
Primer Length: 36  
 $\mu\text{g}$  per OD: 28.5  
nmoles per OD: 2.4  
OD's 28.6  
 $\mu\text{g's}^*$  817  
nmoles 70  
Coupling Eff. 99%

Notes:

\* -See Note about Quantities in  
Supporting Information.

## GIBCO BRL Custom Primers Certificate of Analysis

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Order Date: 09/26/96

**Primer 4:**

Primer Name: KD4

Researcher: DORONIN

Sequence (5' to 3'): GCT CTA GAA GTC AGG CTT CCT GG

Molecular Weight  $\mu\text{g}/\mu\text{mole}$ : 7429.6

Millimolar Extinction Coefficient: 244.0

Purity Deprotected

Tm (1 M Na+) 75

Tm (50 mM Na+) 54

% GC 56

Notes:

Primer Number: A3884G10 (G10)

Primer Length: 23

 $\mu\text{g}$  per OD: 30.4

nmoles per OD: 4.1

OD's 18.6

 $\mu\text{g's}^*$  568

nmoles 76

Coupling Eff. 99%

**Primer 5:**

Primer Name: KD5

Researcher: DORONIN

Sequence (5' to 3'): GCT CTA GAT CTC ATT TAA TCA TA

Molecular Weight  $\mu\text{g}/\mu\text{mole}$ : 7346.6

Millimolar Extinction Coefficient: 251.4

Purity Deprotected

Tm (1 M Na+) 65

Tm (50 mM Na+) 43

% GC 30

Notes:

Primer Number: A3884G11 (G11)

Primer Length: 23

 $\mu\text{g}$  per OD: 29.2

nmoles per OD: 3.9

OD's 17.9

 $\mu\text{g's}^*$  523

nmoles 71

Coupling Eff. 99%

**Primer 6:**

Primer Name: KD6

Researcher: DORONIN

Sequence (5' to 3'): CCT TAA TTA AAA GTC AGG CTT CCT GG

Molecular Weight  $\mu\text{g}/\mu\text{mole}$ : 8370.2

Millimolar Extinction Coefficient: 284.9

Purity Deprotected

Tm (1 M Na+) 73

Tm (50 mM Na+) 51

% GC 42

Notes:

Primer Number: A3884G12 (G12)

Primer Length: 28

 $\mu\text{g}$  per OD: 29.3

nmoles per OD: 3.5

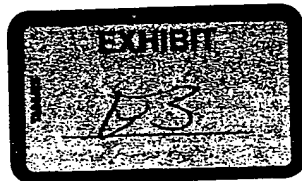
OD's 21.5

 $\mu\text{g's}^*$  634

nmoles 76

Coupling Eff. 99%

\* - See Note about Quantities in  
Supporting Information.



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**GIBCO BRL Custom Primers**  
**Certificate of Analysis****Primer 7:**

Primer Name: KD7  
Researcher: DORONIN  
Sequence (5' to 3'): CCT TAA TTA ATC TCA TTT AAT CAT A  
Molecular Weight  $\mu\text{g}/\mu\text{mole}$ : 7957.0  
Millimolar Extinction Coefficient: 277.0  
Purity Deprotected  
Tm (1 M Na<sup>+</sup>) 63  
Tm (50 mM Na<sup>+</sup>) 41  
% GC 20

Notes:

Primer Number: A3884H01 (H01)  
Primer Length: 25  
 $\mu\text{g}$  per OD: 28.7  
nmoles per OD: 3.6  
OD's 20.3  
 $\mu\text{g's}^*$  586  
nmoles 73  
Coupling Eff. 99%

**Primer 8:**

Primer Name: KD8  
Researcher: DORONIN  
Sequence (5' to 3'): CGC GTA TAC AGA AGA TTT TTC CAG  
Molecular Weight  $\mu\text{g}/\mu\text{mole}$ : 7742.8  
Millimolar Extinction Coefficient: 268.2  
Purity Deprotected  
Tm (1 M Na<sup>+</sup>) 70  
Tm (50 mM Na<sup>+</sup>) 49  
% GC 41

Notes:

Primer Number: A3884H02 (H02)  
Primer Length: 24  
 $\mu\text{g}$  per OD: 28.8  
nmoles per OD: 3.7  
OD's 21.7  
 $\mu\text{g's}^*$  628  
nmoles 81  
Coupling Eff. 99%

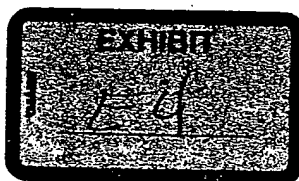
**Primer 9:**

Primer Name: KD9  
Researcher: DORONIN  
Sequence (5' to 3'): CGC GTA TAC ACT GCA GCA GGT GTG  
Molecular Weight  $\mu\text{g}/\mu\text{mole}$ : 7784.8  
Millimolar Extinction Coefficient: 261.8  
Purity Deprotected  
Tm (1 M Na<sup>+</sup>) 77  
Tm (50 mM Na<sup>+</sup>) 56  
% GC 58

Notes:

Primer Number: A3884H03 (H03)  
Primer Length: 24  
 $\mu\text{g}$  per OD: 29.7  
nmoles per OD: 3.8  
OD's 20.5  
 $\mu\text{g's}^*$  609  
nmoles 78  
Coupling Eff. 99%

\* -See Note about Quantities in  
Supporting Information.

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FROM : SAINT LOUIS UNIVERSITY MMI

FAX NO. : 3147733403

Nov. 26 2002 06:02PM P6

|  |            |          |
|--|------------|----------|
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| 522393 A                                 | 11/04/96   | 1 of 1   |

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000127752

|     |         |                     |
|-----|---------|---------------------|
| CEH | S903851 | FAX/DEBBIE HUMISTON |
|-----|---------|---------------------|

|     |           |                       |        |         |      |
|-----|-----------|-----------------------|--------|---------|------|
| 1.0 | 10338-022 | KD 10 \ DORANIN       | 1 Each | ( B02 ) | 12.6 |
|     | A4379B02  | ACACGGCACCAGCTCAATCAG |        |         |      |
| 2.0 | 10336-022 | KD 11 \ DORANIN       | 1 Each | ( B03 ) | 12.5 |
|     | A4379B03  | CGGAGTAACTTGATGTGTTG  |        |         |      |





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Order Number: 522393A

Order Date: 11/04/96

**GIBCO BRL Custom Primers**  
**Certificate of Analysis****Primer 1:**

Primer Name: KD 10  
Researcher: DORANIN  
Sequence (5' to 3'): ACA CGG CAC CAG CTC AAT CAG  
Molecular Weight  $\mu\text{g}/\mu\text{mole}$ : 6709.2  
Millimolar Extinction Coefficient: 232.1  
Purity **Deprotected**  
Tm (1 M Na+) 73  
Tm (50 mM Na+) 51  
% GC 57

Notes:

Primer Number: A4379B02 (B02)  
Primer Length: 21  
 $\mu\text{g}$  per OD: 28.9  
nmoles per OD: 4.3  
OD's 12.6  
 $\mu\text{g's}^*$  366  
nmoles 54  
Coupling Eff. 98%

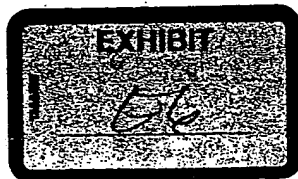
**Primer 2:**

Primer Name: KD 11  
Researcher: DORANIN  
Sequence (5' to 3'): CGG AGT AAC TTG TAT GTG TTG  
Molecular Weight  $\mu\text{g}/\mu\text{mole}$ : 6847.2  
Millimolar Extinction Coefficient: 233.0  
Purity **Deprotected**  
Tm (1 M Na+) 67  
Tm (50 mM Na+) 45  
% GC 42

Notes:

Primer Number: A4379B03 (B03)  
Primer Length: 21  
 $\mu\text{g}$  per OD: 29.3  
nmoles per OD: 4.2  
OD's 12.5  
 $\mu\text{g's}^*$  369  
nmoles 53  
Coupling Eff. 98%

\* -See Note about Quantities in  
Supporting Information.

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|   |            |          |
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| 409867 A                                  | 03/24/97   | 1 of 1   |

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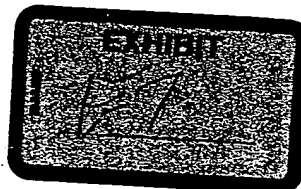
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| CD | S903850      |          |             |     |            | DEBBIE HUMSTON |

|     |           |                                  |        |         |      |
|-----|-----------|----------------------------------|--------|---------|------|
| 1.0 | 10336-022 | KD12 \ DORONIN                   | 1 Each | ( D10 ) | 16.4 |
|     | A6280D10  | CCTTAATTAATCTAGAGATCTTATTCCTTT   |        |         |      |
| 2.0 | 10336-022 | KD14 \ DORONIN                   | 1 Each | ( D11 ) | 14.7 |
|     | A6280D11  | GGGGTACGAAGCCATCTGCAACAACAT      |        |         |      |
| 3.0 | 10336-022 | DK15 \ DORONIN                   | 1 Each | ( D12 ) | 13.7 |
|     | A6280D12  | CCTTAATTAATCTAGAGTCAGTTAGCCTCCCC |        |         |      |
| 4.0 | 10336-022 | KD16 \ DORONIN                   | 1 Each | ( E01 ) | 14.6 |
|     | A6280E01  | CGCGCGTATACACTTCCCATTTTAAG       |        |         |      |
| 5.0 | 10336-022 | KD17 \ DORONIN                   | 1 Each | ( E02 ) | 12.9 |
|     | A6280E02  | GCTCTAGACATCATCAATAATAT          |        |         |      |



ST LOUIS UNIV SCHOOL OF

Order Number: 409867A

Order Date: 03/24/97

**GIBCO BRL Custom Primers**  
*Certificate of Analysis***Primer 4:**

Primer Name: KD16  
Researcher: DORONIN  
Sequence (5' to 3'): CGC GCG TAT ACA CTT CCC ATT TTA AG  
Molecular Weight  $\mu\text{g}/\mu\text{mole}$ : 8306.2  
Millimolar Extinction Coefficient: 272.6  
Purity Deprotected  
Tm (1 M Na+) 74  
Tm (50 mM Na+) 53  
% GC 46

Notes:

Primer Number: A6280E01 (E01)  
Primer Length: 26  
 $\mu\text{g}$  per OD: 30.4  
nmoles per OD: 3.6  
OD's 14.5  
 $\mu\text{g's}^*$  444  
nmoles 53  
Coupling Eff. 99%

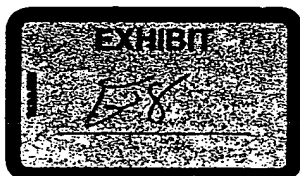
**Primer 5:**

Primer Name: KD17  
Researcher: DORONIN  
Sequence (5' to 3'): GCT CTA GAC ATC ATC AAT AAT AT  
Molecular Weight  $\mu\text{g}/\mu\text{mole}$ : 7364.6  
Millimolar Extinction Coefficient: 263.4  
Purity Deprotected  
Tm (1 M Na+) 66  
Tm (50 mM Na+) 43  
% GC 30

Notes:

Primer Number: A6280E02 (E02)  
Primer Length: 23  
 $\mu\text{g}$  per OD: 27.9  
nmoles per OD: 3.8  
OD's 12.9  
 $\mu\text{g's}^*$  361  
nmoles 49  
Coupling Eff. 99%

\*-See Note about Quantities in  
Supporting Information.

LIFE  TECHNOLOGIES.

## Primers:

1) KD1 - CTA'CGA'GAG'AAC'CTC'TCC'GAG'

Comment - primer from Ad5 sequence, 50' at left from SmaI site -  
for PCR mutation of gp13K (E3) - Left primer.

2) KD2 - GCC'ACA'ACT'TAT'ACT'GTT'TCC

Comment - primer from Ad5 sequence, 50' at right from Bst1107I site  
- for PCR mutation of gp13K (E3) - right primer.

3) KD3 - TCA'GCC'CAC'GGT'ACT'(TAA'TTAA)CC'CAA'AAG'GTG'GA

Comment - middle primer for mutation of gp13K E3 protein gene.  
inserting PacI site with TAA stop codon into the ORF  
destroying KpnI site simultaneously, to be used 1 with 'KD2',  
then product used as primer with 'KD1'.

4) KD4 - GC'TCT'AGA'AGT'CAG'GCT'TCC'TGG

Comment - left primer for PCR of 11.6K E3 with XbaI site.

5) KD5 - GC'TCT'AGA'TCT'CAT'TTA'ATC'ATA

Comment - right primer for PCR of 11.6K E3 with XbaI site.

6) KD6 - CC'TTAATTAA'GT'CAG'GCT'TCC'TGG

Comment - left primer for PCR of 11.6K E3 with PacI site

7) KD7 - CC'TTAATTAA'TCT'CAT'TTA'ATC'ATA

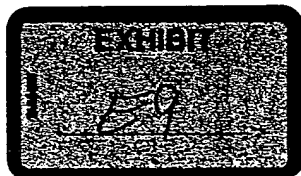
Comment - right primer for PCR of 11.6K E3 with PacI site.

8) KD8 - CGG'GTATAC'AGA'AGATT'TTTC'CAG'

upstream primer with Bst1107I site for PCR of SP-B 500 promoter.

9) KD9 - CGC'GTATAC'ACT'GCA'GCA'GGT'GTC

downstream primer with Bst1107I site for PCR of SP-B 500 promoter.

22-141 50 SHEETS  
22-142 100 SHEETS  
22-144 200 SHEETS

Primers for sequencing  $\Delta E4$  substitutions ;  
promoter.

- 10) K1 (10): ACA'CGG'CAC'GAG'CTC'AAT'GAG' (left)  
11) K1 (11): CCG'AGT'AAC'TTG'TAT'GTG'TTG (right)

Primers for TK insert into Pst and Xba sites. (double PCR,  
12+14, then product with 15  
on Ad template).

CC  
~~CC~~ TTAATTAA TCTAGA GAT'CTT'ATT'CCC'TTT

Primer with Pst and Xba sites + Ad seq 30818 →  
upstream primer.

14) K1 (14)

GGG'GTA'CGA'AGC'CAT'CTG'CAA'CAA'CAT

Primer left half from 5' tkorf, right half - from Ad ~ 31000  
→ beginning of fiber gene.  
to prevent methylation.

15) CC TTAATTAA TCTAGA G TCA'GTT'AGC'CTC'CCC  
(3)

Primers to PCR Ad5 right ITR (Ad. 35773-35935) with  
flanking bst1107I and XbaI sites. respectively.

16) CCGCC GTATAC ACT'TCC'CAT'TTT'AAG

17) GCTCTAGA CAT'CAT'CAA'TAA'TAT

18) 18 - Direct - GTA-GAG-TTT-TCT-CCT-CCG  
for PCR and sequence of 1101/1107 dl.

19) 19 - Reverse - CCC-TCT-TCA-TCC-TCC-TCC

20 - TCT,ACT,TTA,ACC,CCT,TCT,CCG CE3 direct to K1 dl)

21 - CCG,AGG,TGT,TAT,TAC,CGA,AGA CE1A, M01 dl)

22 - TGA,CGT,AAC,CGT,TAA,AGT,CCA (from E4 prom dl)  
direct

50 SHEETS  
22-141 100 SHEETS  
22-142 200 SHEETS  
22-144



## **EXHIBIT F**

Page 1 of 1

Nov 19, 1986 2:40 PM

Nov 19, 1996 11:42 AM

Spacing: 13.83 ABI-CE2 to 2.1.2

Signal G:340 A:481 T:305 C:253

DT POP6 10-10-96

POP6DT.Matrix

**Points 2080 to 10200 Base 1: 2080**

## Box Chrome I

**A1-KD**

$$T(x)T(x)$$

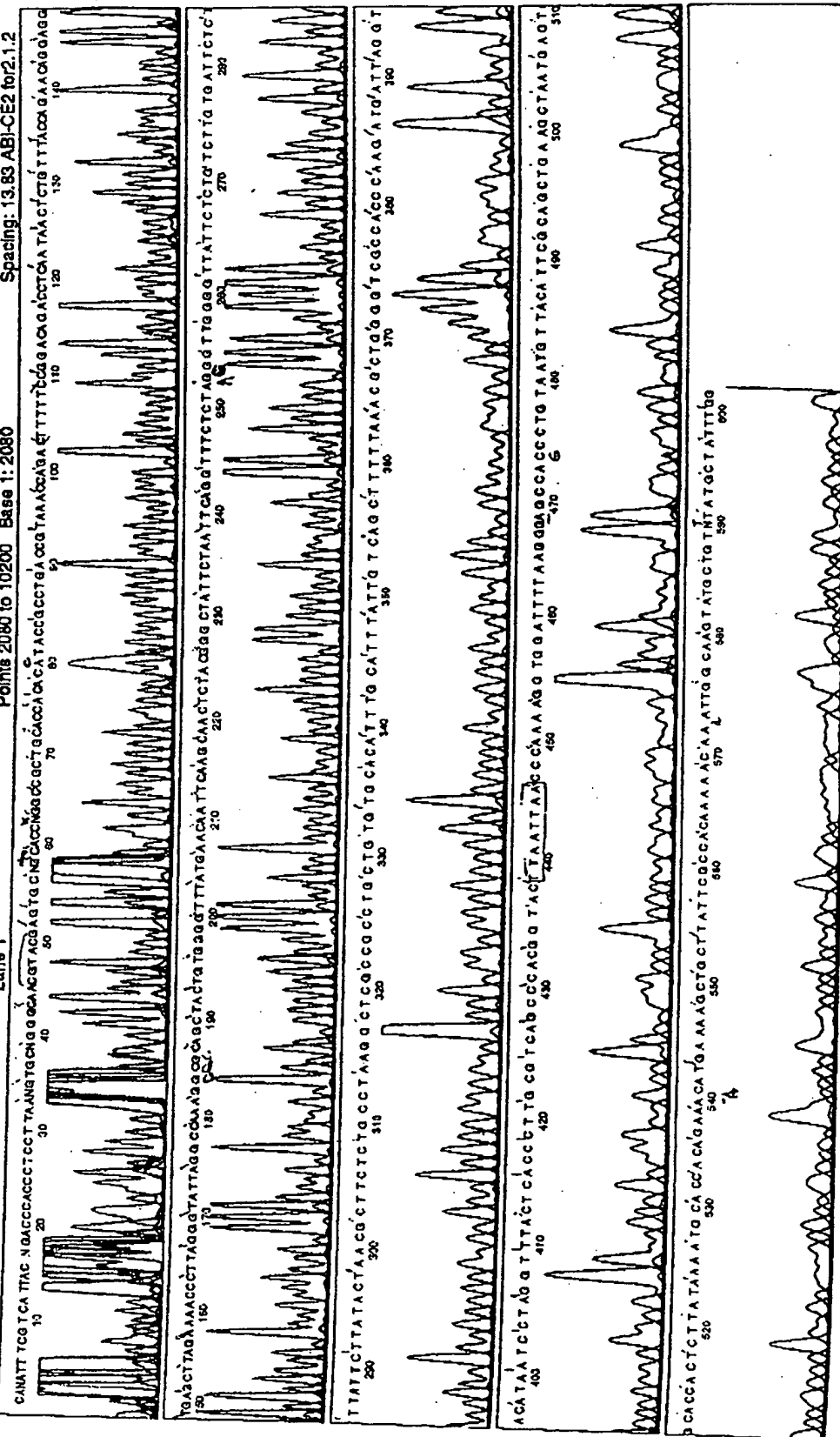
KD.

**Lane 1**

**Model 310-3**  
**Version 2.1.2**

**Version 2.1.2**

**ABI PRISM**

**EXHIBIT**

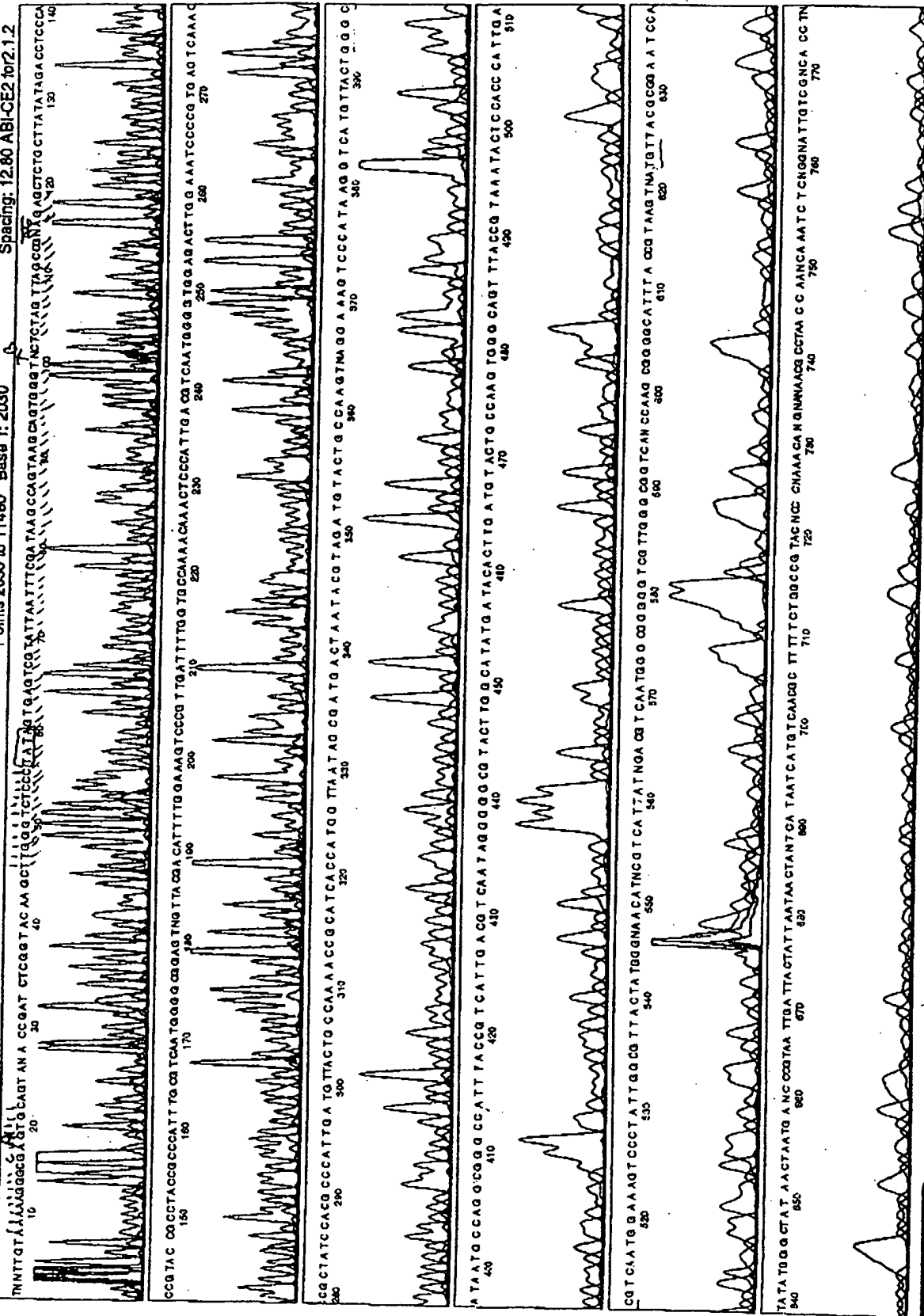








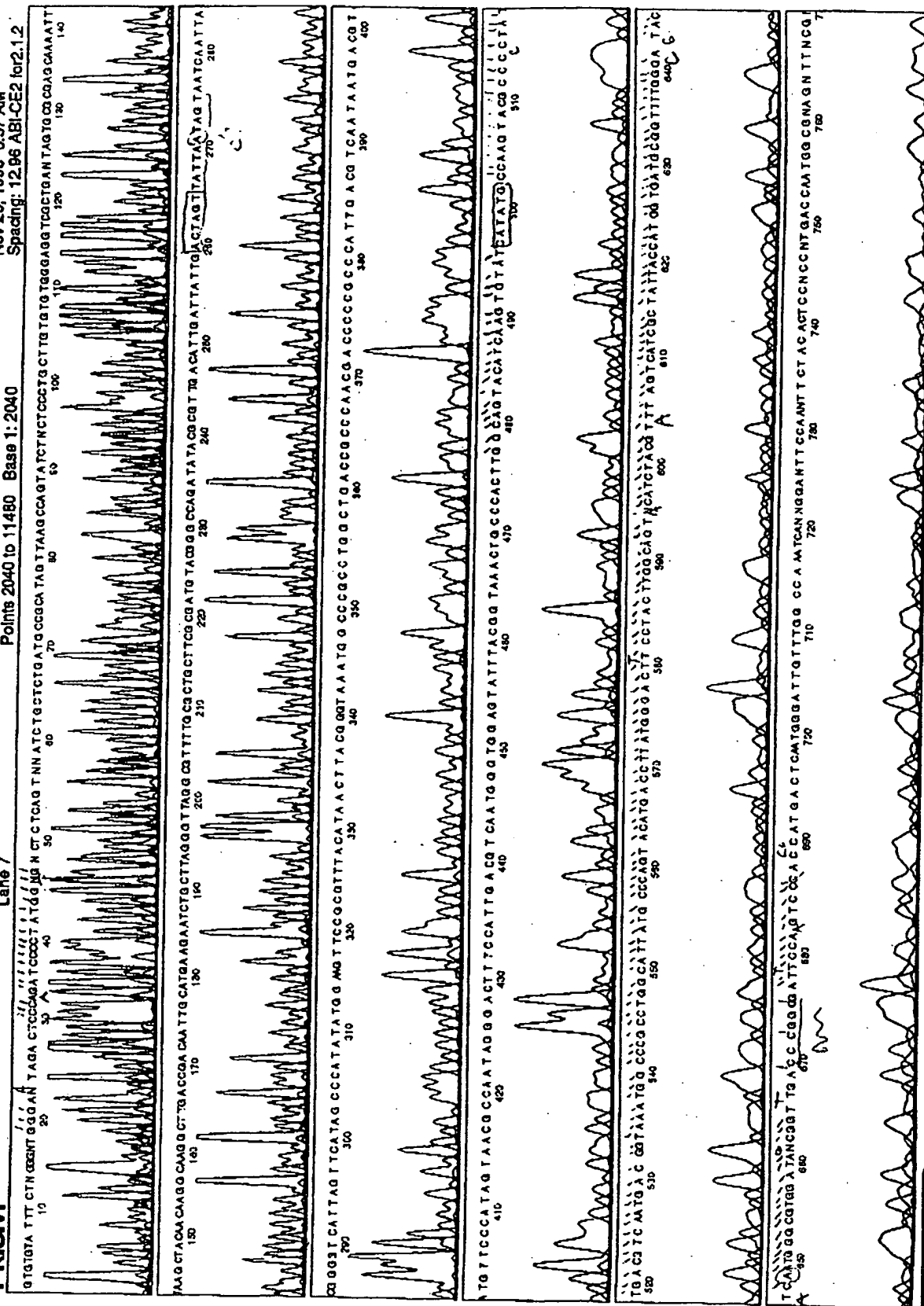
Nov 28, 1996 9:53 AM  
Nov 26, 1996 4:08 AM



**四、设备**

Spading: 12.96 ABI-CE2 for 2.1.2

yes, clade Z3, primer cD11.



# EXHIBIT

11

Model 310  
Version 2.1.2

B4-KD-#7

KD-#7

Lane 8

Signal G:176 A:383 T:218 C:180

DT POP6 10-10-98

POP6DT.Mattx

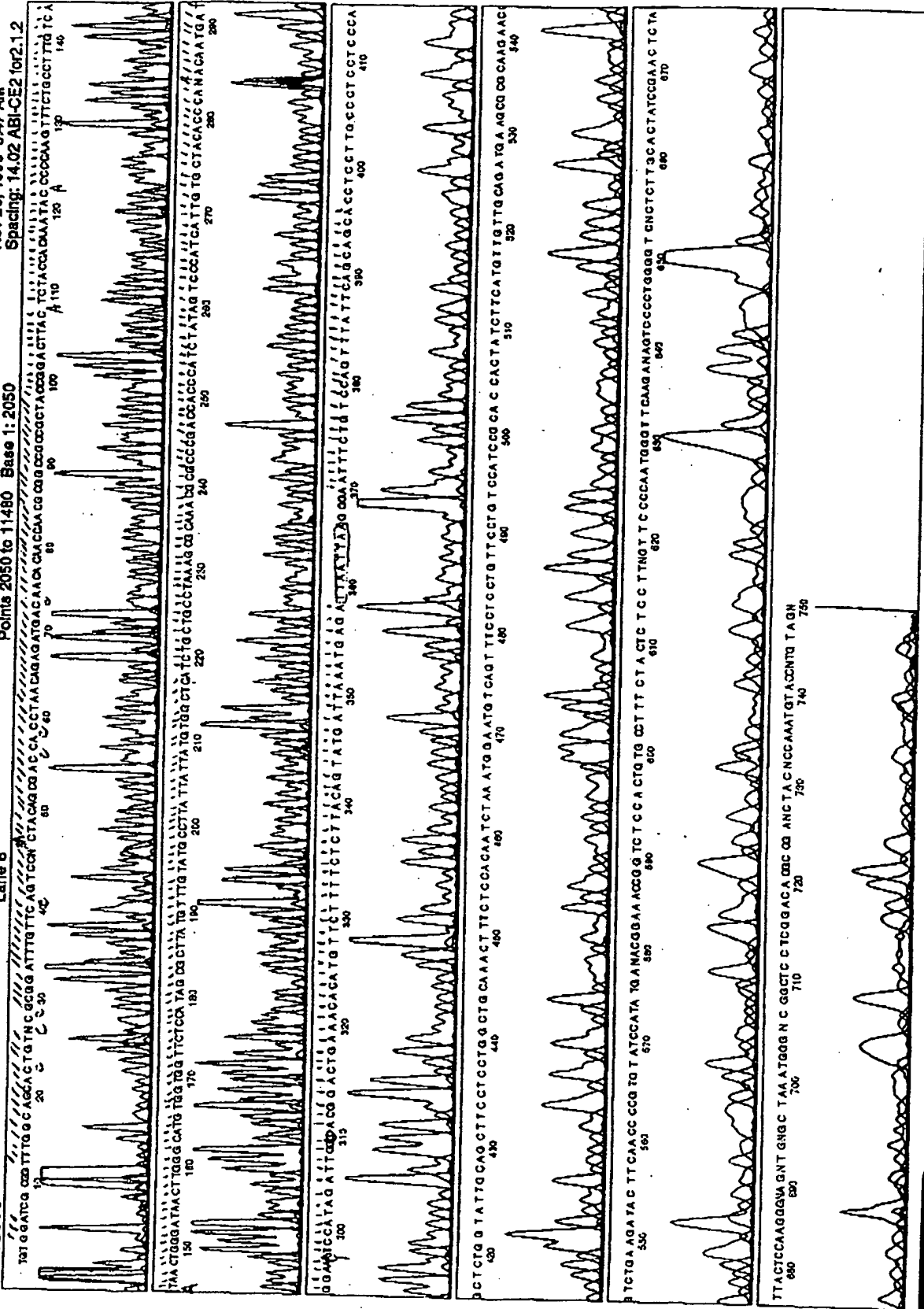
Points 2050 to 11480 Base 1: 2050

Page 1 of 1

Nov 26, 1996 1:21 PM

Nov 26, 1996 8:47 AM

Splicing: 14.02 ABI-CE2 for 2.1.2



EXHIBIT

F7



ABI  
PRISM

Model 310  
Version 3.0  
ABI-CE2 3.0  
Version 3.0b1

A1-Wold-9  
Wold-9  
Lane 1

(p21, primer 4310)

Signal G:151 A:256 T:158 C:157  
DT POP8  
pop8 dt-sep-ite-stds.mtrix  
Points 844 to 9360 Base 1: 944

Page 1 of 1  
Thu, Mar 20, 1997 7:47 PM  
Thu, Mar 20, 1997 5:02 PM  
Spacing: 11.90(11.90)

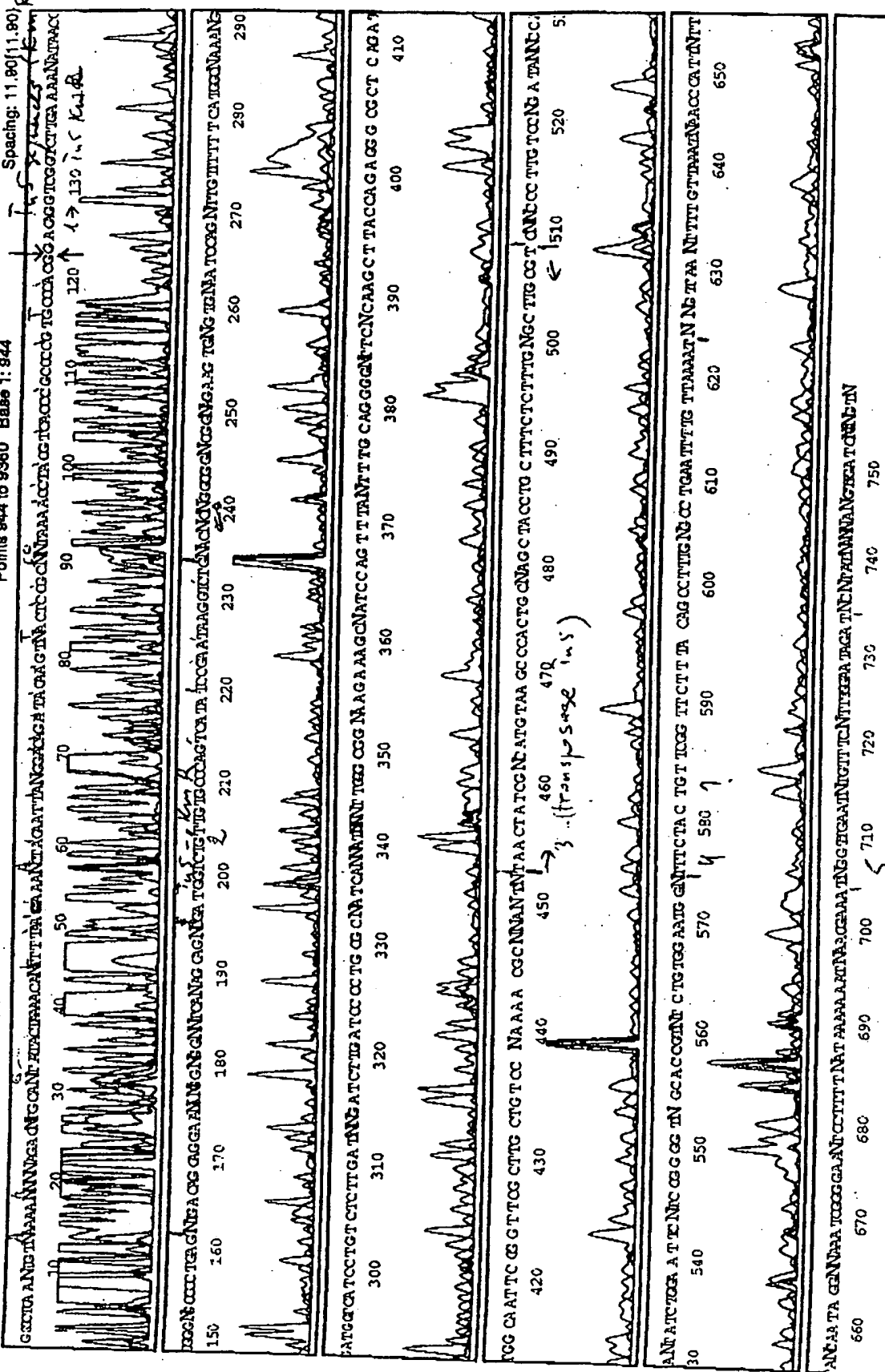
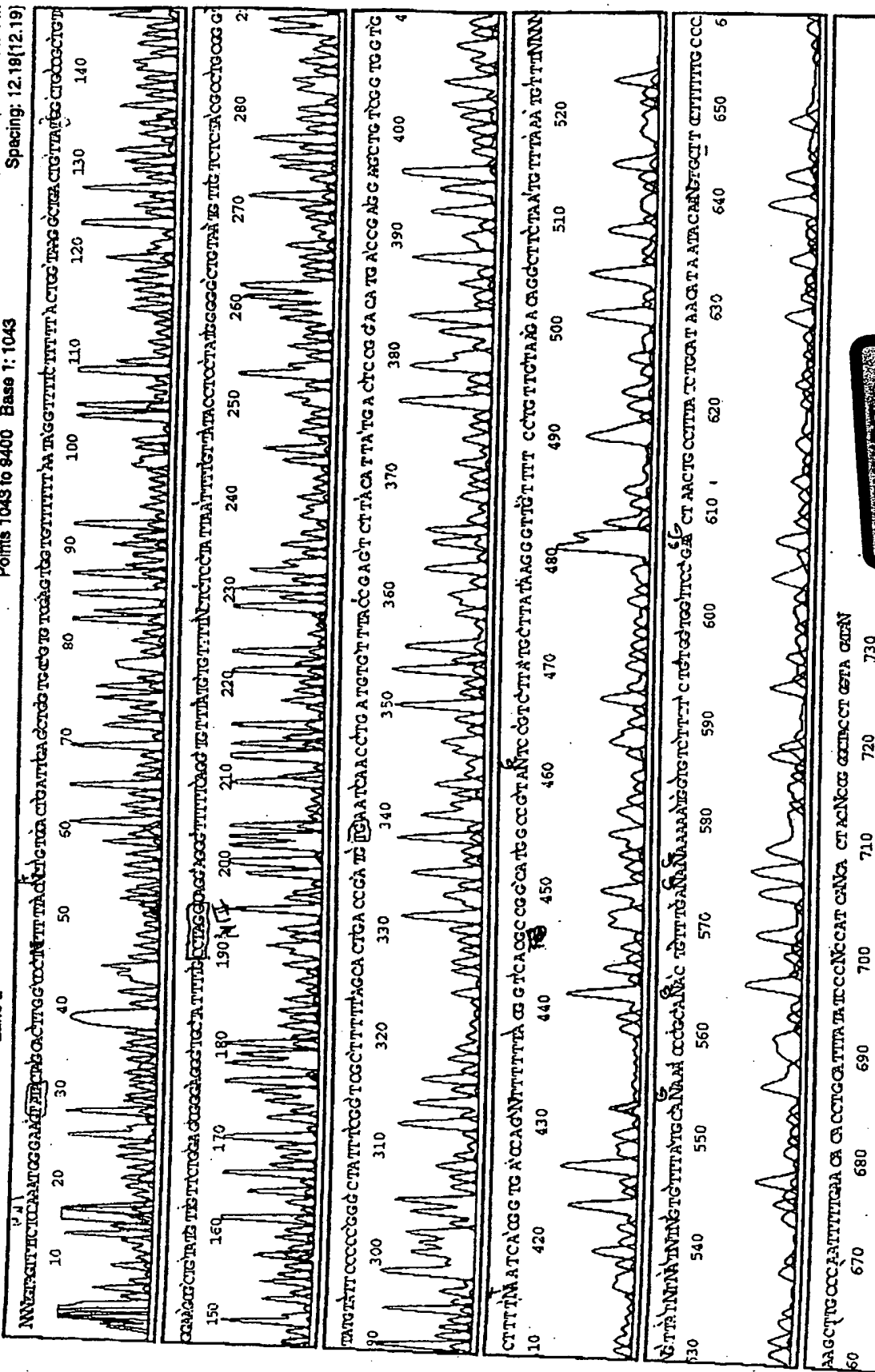


EXHIBIT  
29

Signal G:639 A:658 T:543 C:294  
DT POP8  
pop8 dt-septe-stds.mtrix  
Points 1043 to 9400 Base 1: 104

Page 1 of 1  
Thu, Mar 20, 1997 10:22 PM  
Thu, Mar 20, 1997 7:47 PM  
Spacing: 12.18{12.19}



EXHIBIT

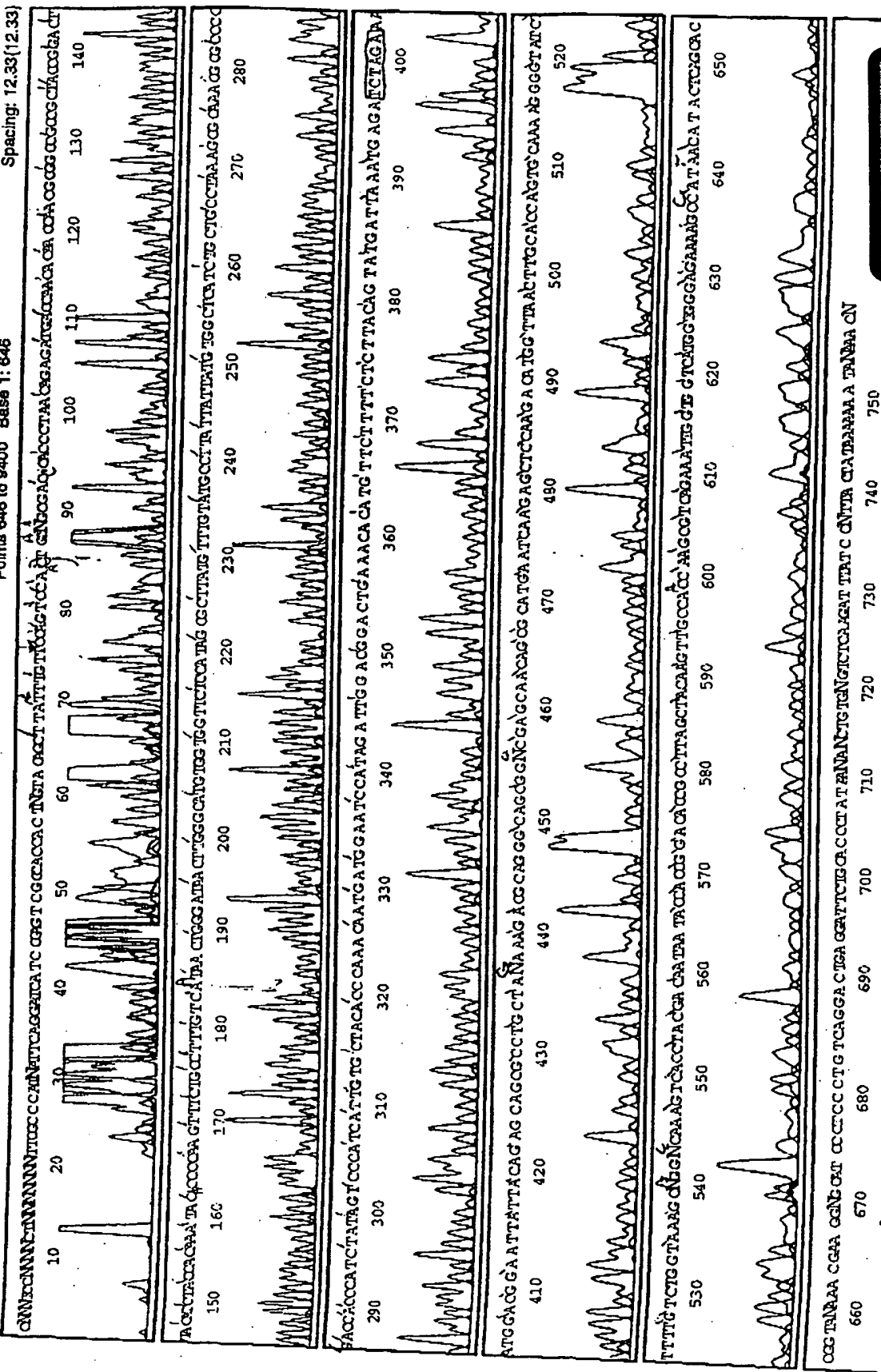
1

7-10



Signal G:278 A:419 T:211 C:190  
DT POP8  
pop8 dt-seprie-stds.mtx  
Points 646 to 9400 Base 1: 646

Page 1 of 1  
Fri, Mar 21, 1997 12:57 AM  
Thu, Mar 20, 1997 10:22 PM  
Spacing: 12.33(12.33)



上海工人



**ABI PRISM**  
Model 310  
Version 3.0  
ABI-CE2 3.0  
Version 3.0b1

A1-KostyaWold-14

KostyaWold-14  
Lane 1

Signal G:71 A:84 T:75 C:57

DT POPE

pop8 dt-seprte-sids.mrx

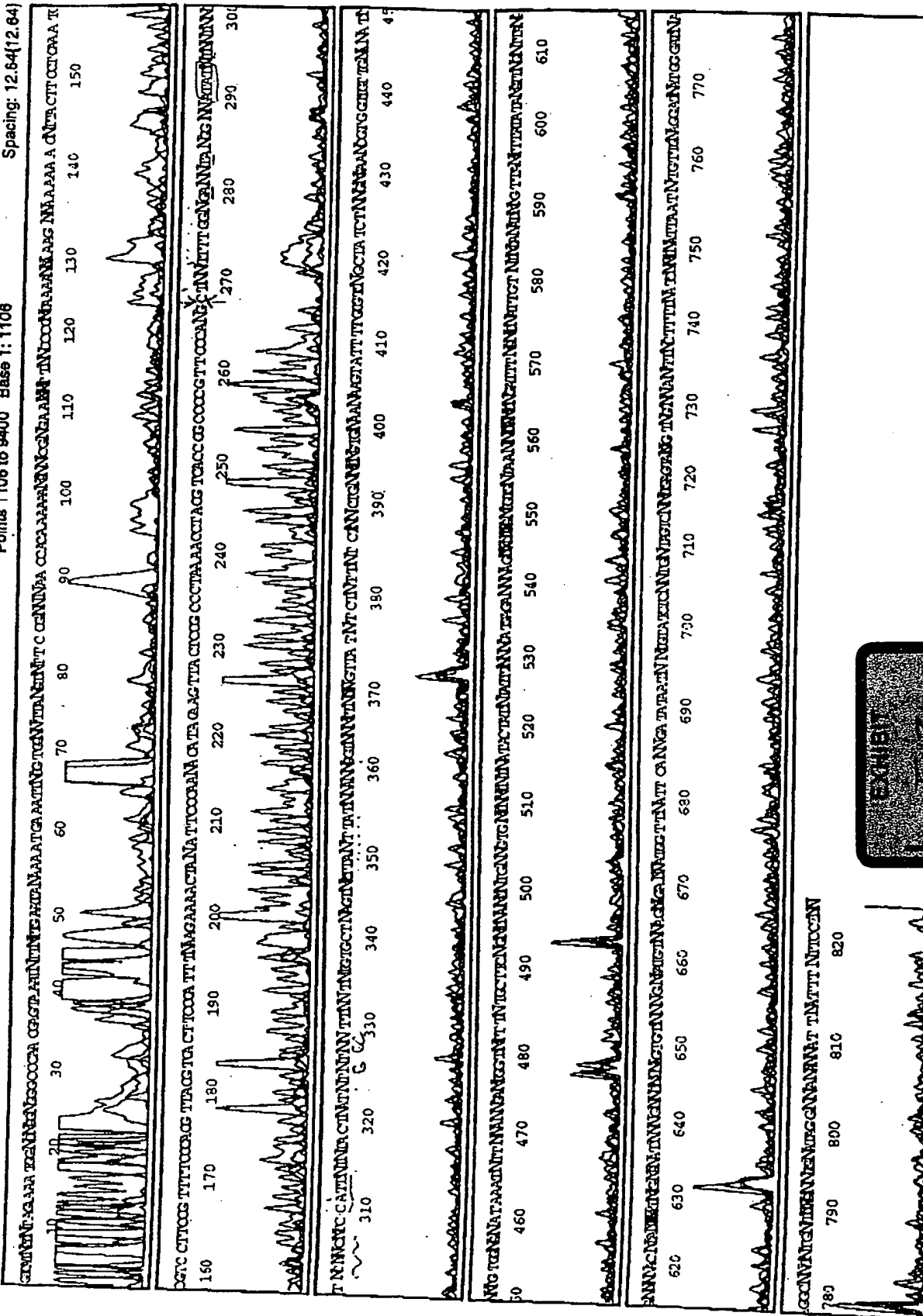
Points 1106 to 9400 Base 1: 1108

Page 1 of 1

Mon, Apr 21, 1997 12:49 PM

Mon, Apr 21, 1997 9:22 AM

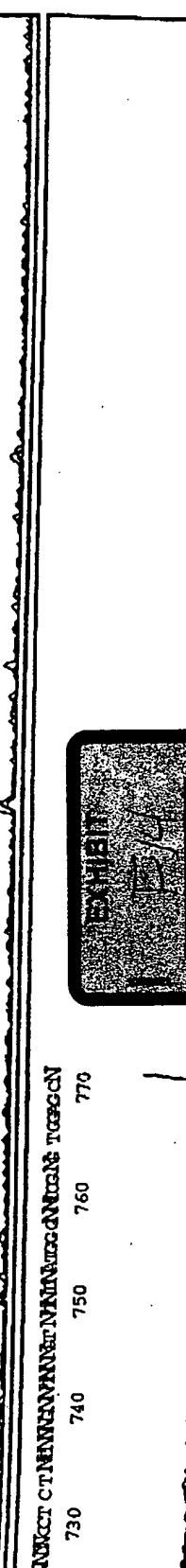
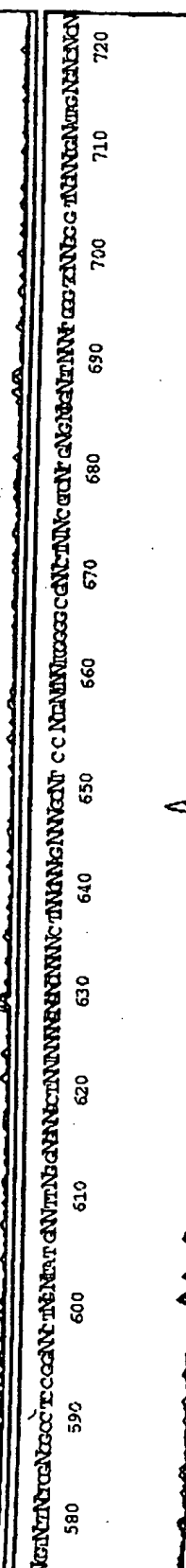
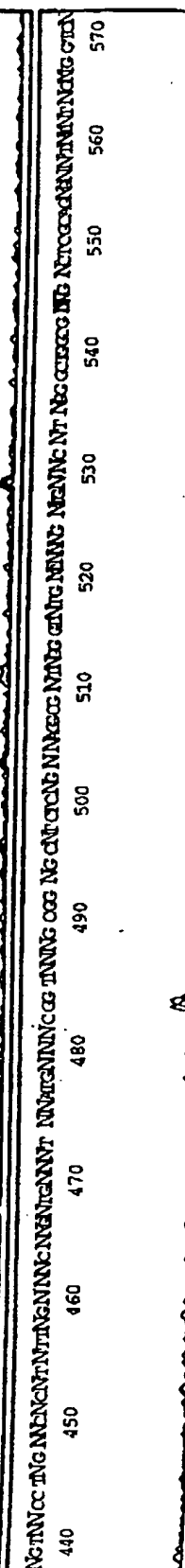
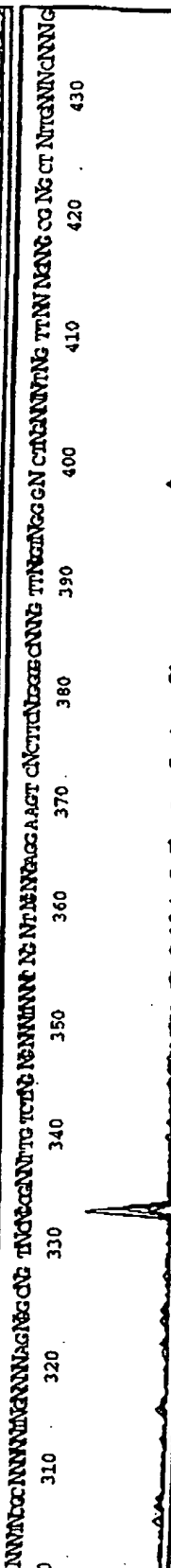
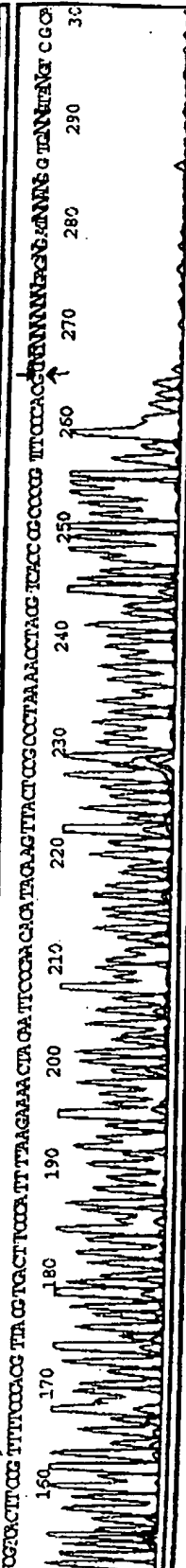
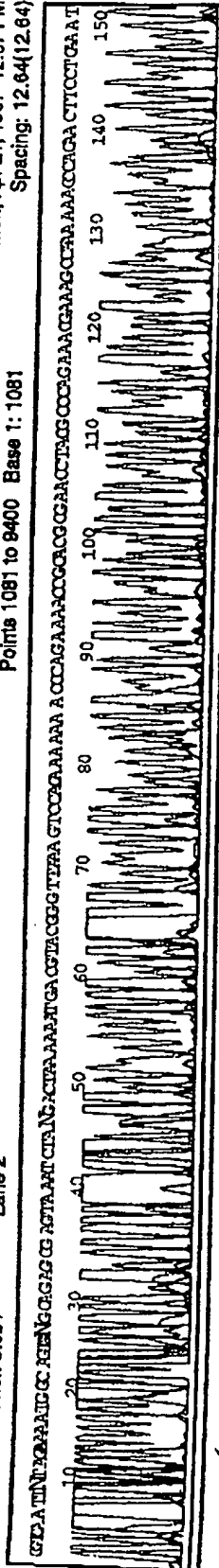
Spacing: 12.64(12.64)




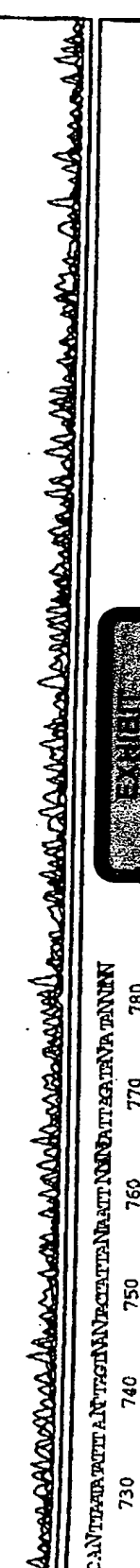
р. В. Г. 10,  
принимать 10.

Signal G:151 A:330 T:175 C:161  
DT POP8  
pop6 dt-septre-stds.mtrix  
Points 1081 to 9400 Base 1: 1081

Page 1 of 1  
Mon, Apr 21, 1997 2:42 PM  
Mon, Apr 21, 1997 12:07 PM  
Spacing: 12.64(12.64)



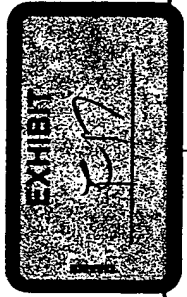
Spacing: 12.48{12.48}

A small, rectangular, textured label with the word "EXHIBIT" printed vertically on the left side. In the center, the number "15" is written above the letter "A", both enclosed within a hand-drawn rectangular border.

Substance Abuse



Page 1 of 1  
97 10:27 PM  
97 7:52 PM  
2.48{12.48}



0000NKGAT TC NBT NWCCNNNTN CNGNTANA ANVNTNNNN  
650 660 670 680 690



**Lane 8**

Mon, May 12, 1997 10:24 AM  
Mon, May 12, 1997 7:49 AM  
Spacing: 12.64{12.64}

Mon, May 12, 1997 10:24 AM

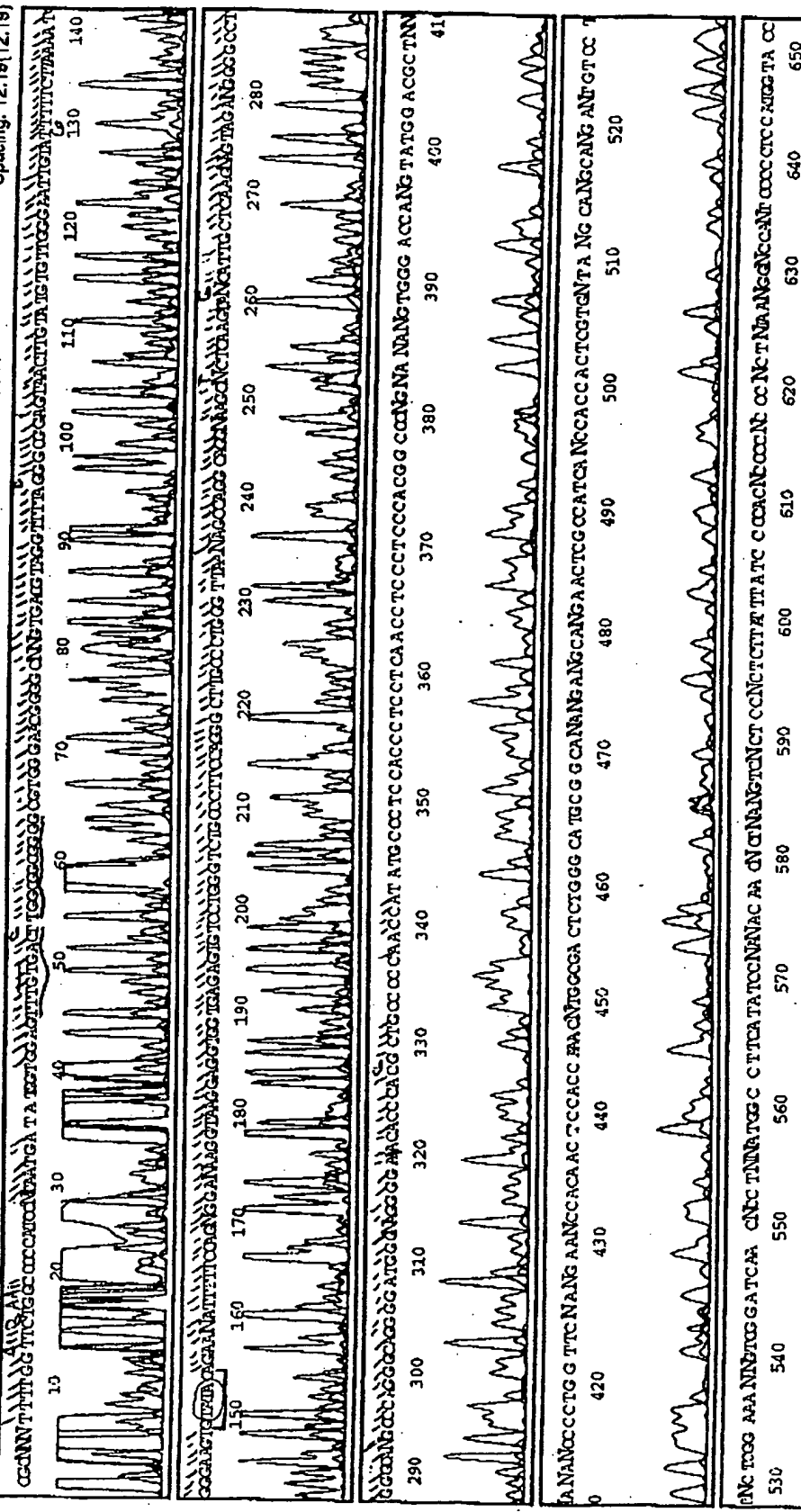
Mon, May 12, 1997 7:49 AM



8/7



Mon, May 12, 1997 1:00 PM  
Mon, May 12, 1997 10:24 AM  
Spacing: 12.19{12.19}



EXHIBIT

5/2

**ABI PRISM**

PCP product  
1101/1107 6818-19

Sequence 1107 WD18-19  
1107 WD18-19  
Sequence 1107 WD18-19

Signal G:1955 A:1439 T:1255 C:1906  
DT POP6(BD Set-Any Primer)  
dRho22

Page 1 of 1  
Tue, Mar 23, 1999 10:45 F  
Tue, Mar 23, 1999 8:00 P  
Spacing: 12 pt

Lane 5  
Sequence → primer 018 (direct) dRhod2

Tue, Mar 23, 1999 10:45 F Page 1 of 1  
Tue, Mar 23, 1999 8:00 P

DT POP(BD Set-Any Primer)

Points 1100 to 10200 Base 1: 1100 Spacing: 12,19|12,11

GTGTCGCCCGGAATGAATTGAGAAATGAGTACGTGGCTTGATAATCCTTCACCACTTCCACCCTTCCGAC TGTATG

10 20

6' 30' 40' 50' 60' 70' 80'

ATTTAGACGCGCC<sup>100</sup>CCGAAGATCCCAACGANN<sup>120</sup>GANA<sup>130</sup>GCGGT<sup>140</sup>TTCGCA<sup>150</sup>ATTTT<sup>160</sup>TCCGAC<sup>170</sup>TCTGTAA<sup>180</sup>TGTTGCGG<sup>190</sup>GT<sup>200</sup>

GCAGGSA A WNGATTG AG TTAC TCAC TTT TCC GCC GCC CC GG T TCT TCC GAGG GGC TCCAG T TCCAGG GGCAGG

CC GAGG A G C C G G A G C A G A G A G C T T G G G T C C G G T T T G C C A C G A G G C T G G G T T T C C A C C C A G T G A C G A C G A G A

320

310

330

340

350

360

370

380

390

400

410

420


430

440

450

EXHIBIT

**EXHIBIT**





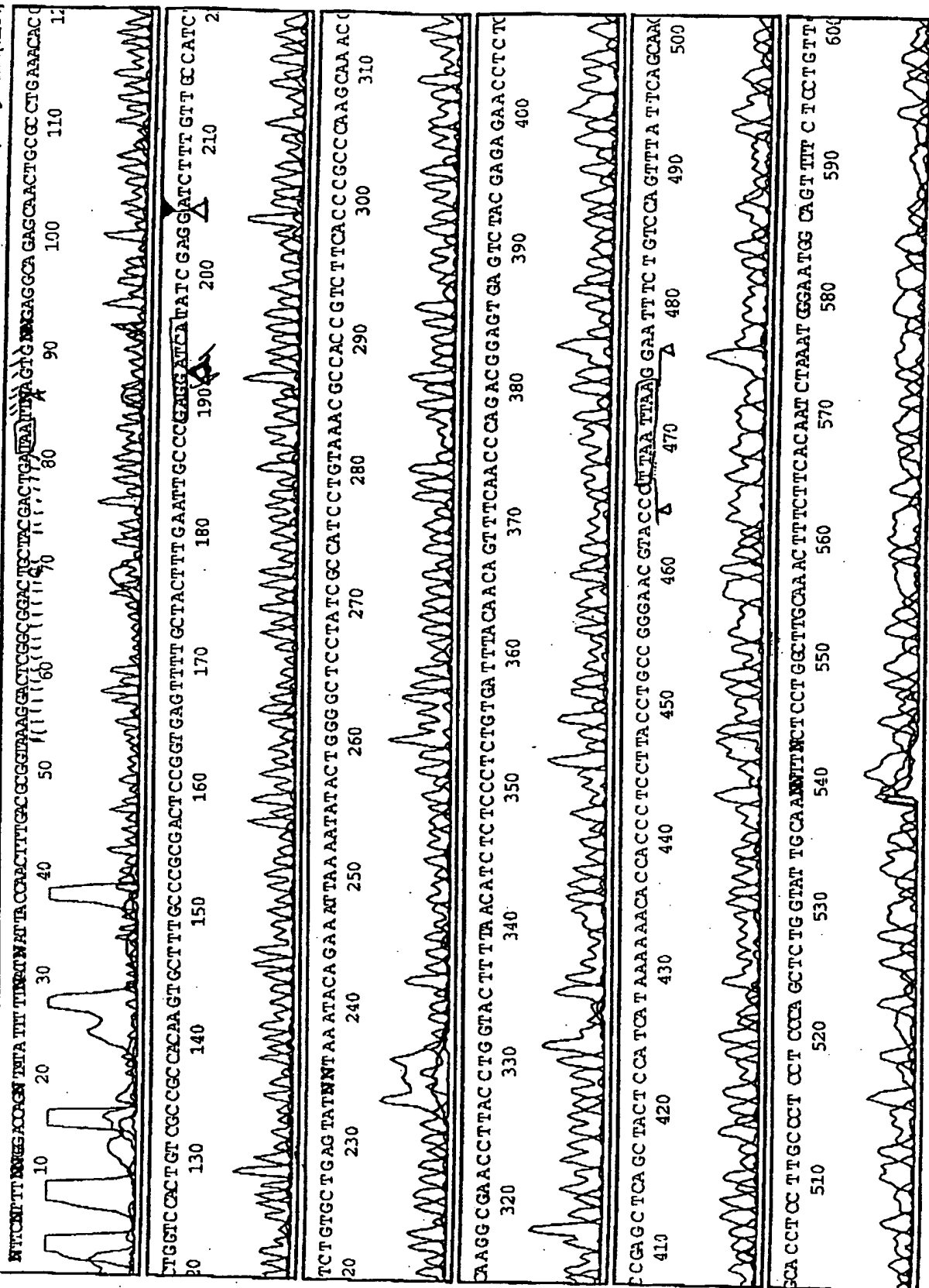




Page 1 of 2  
Mar 24, 1999 5:15 PM  
Mar 24, 1999 2:30 PM  
Spacing: 12.04/12.04}

# EXHIBIT

421





**ABI PRISM**

Model 310  
Version 3.0  
ABI-CE1  
Version 3.0

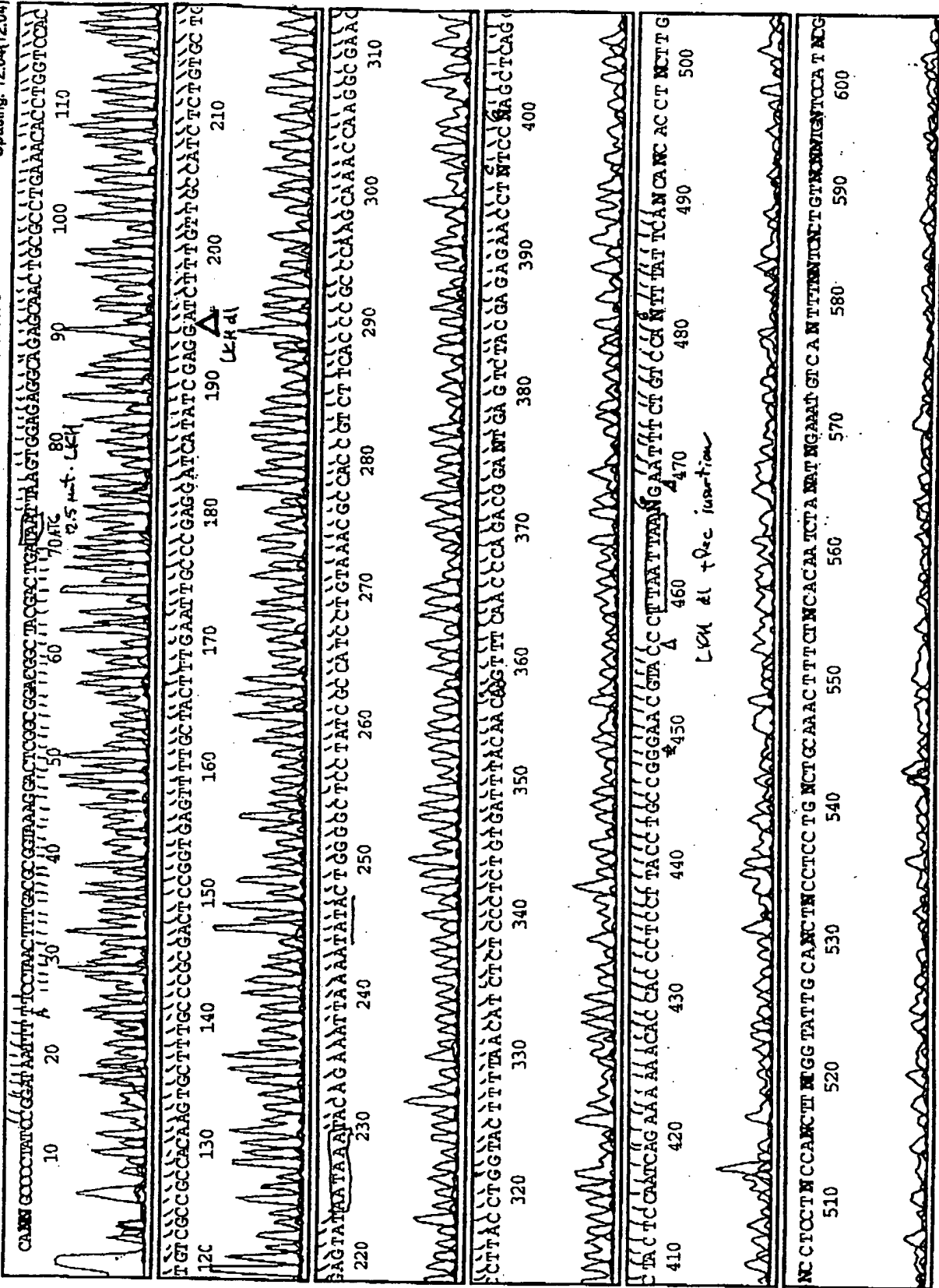
Donorin #8  
Donorin #8  
Lane 3

pl2, alkline denaturation  
primer 0920 (Dir)

Signal G:65 A:41 T:42 C:53  
DT POP8BD Set-Any Primer  
dRhod2

Page 1 of 2  
24, 1999 8:01 PM  
Wed, Mar 24, 1999 5:15 PM  
Spacing: 12.04(12.04)

Points 1076 to 10200 Base 1: 1076



## **EXHIBIT H**



970619 Infection of A549 (Kostya's Virus)

A549 (6/18) "1-40" - ~80% confl. (estimate  $10^6$  cells/TP)

Added SF-DME (2ml), removed & added 1ml SF-DME (~11Am);

Infection at 2:30 pm

| <u>Virus</u>                               | <u>Prep</u>               | <u>Titer</u>                   | <u>Inf. Volume</u><br><u>(1:20 dilution)</u> | <u># TP</u>       |
|--|---------------------------|--------------------------------|--|-------------------|
| ① mock                                     | —                         | —                              | —  | 1                 |
| ② dl 309 (VS 119) (970308)                 | —                         | $1.2 \times 10^{11}$           | 10 $\mu$ l/TP                                | 4                 |
| ③ KD-1 <sup>(1101/1107)</sup> ADP (970508) | —                         | $9.64 \times 10^{10}$          | 15 $\mu$ l/TP                                | 4                 |
| ④ KD-2 (970611)                            | —                         | estim $4-5 \times 10^{10}$     | 20 $\mu$ l/TP                                | 4                 |
| ⑤ KD-3 970617; <sup>~15 ml</sup> 32g sup   | —                         | estimated $2-3 \times 10^{10}$ | { 25 $\mu$ l/TP<br>40 $\mu$ l/TP             | 3<br>1 (Ara-C TP) |
|  | 150 cm <sup>2</sup> flask |                                |  |                   |

Added 1/2 ml DME (10% FCS) at 3:30 pm.

Added 4X Ara-C<sup>(10  $\mu$ l of stock)</sup> at ~6 pm & again at 9 AM on (6/20), fixed Ara-C & one set of coverslips at 27h post-infr. (other ss's fixed at 49 hours post-infection → all fixed in Paraformaldehyde followed by MeOH/DAPI, then a 2 min MeOH rinse & rehydrated in PBS; stained on (6/23) [after checked out Baolings antibodies]

EXHIBIT

4/1

970623

## Immunofluorescence Staining

all fixed in PF/meth to DAPI

Set #1

(M73(1:2)

121531 (1:1000 fine)

600  $\mu$ l

- 1 mock (Ara-C) slight backgrd (all cells)
- 2 dl 309 (Ara-C) 400% inf'd; some Golgi
- 3 dl 309 (27h) Golgi + speckled around nuclei
- 4 KD-1 (Ara-C) 80-90% inf'd; some Golgi
- 5 KD-1 (27h) much more than Ads; very vesicular
- 6  $\downarrow$  (49h) all cells bright (all vesicular)
- 7 KD-2 (Ara-C) 70-80% inf'd; some Golgi
- 8  $\downarrow$  (27h) more like Ads, but some nuclei staining
- 9  $\downarrow$  (49h) more diffuse; some cells quite bright; some vesicles
- 10 KD-3 (Ara-C) 50-60% inf'd; pretty similar to Ads (Ara-C)
- 11  $\downarrow$  (27h) not too much staining
- 12  $\downarrow$  (49h) some cells brighter (again rather "diffuse")

Set #2 ( $\alpha$ DBP /  $\alpha$ Fib, each 1:400)250  $\mu$ l

- 13 mock (49h) slight backgrd (esp. FITC)
- 14 309 (27h) all nuclei stain for both
- 15 KD-1 (49h) strong staining for both
- 16 KD-2  $\downarrow$  more stained for DBP
- 17 KD-3  $\downarrow$  fewer cells stained for Fib

A-549 (6/18) 1:40 (p100) - Inf'd (6/19)  
 volume of 1: dil: 309 (10  $\mu$ l), KD-1 (15  $\mu$ l), KD-2 (20  $\mu$ l), KD-3 (40  $\mu$ l (Ara-C), or 25  $\mu$ l)

1st Ab(s): 50  $\mu$ l for 60 minutes;

PBS rinses: 70 min, 12 min;

2nd Ab(s): (970612) Goat  $\alpha$  rabbit FITC - & Goat  $\alpha$  Mouse - FITC (not preadsorbed)  
 1:50 dilution of each  
 50  $\mu$ l for 34 minutes;

PBS rinses: 6 1/2 min, 5 min;

dd H<sub>2</sub>O dip

mounted in Elvanol in p-phenylenediamine

EXHIBIT

#2

970(5/20) Plaque Assays (New Counts)

| Virus    | Dilution             | Dish | 5/26 | 5/28 | 5/30 | 6/2 | 6/4 | 6/6 | 6/8  |
|----------|----------------------|------|------|------|------|-----|-----|-----|------|
| ① dl 751 | $.5 \times 10^{-8}$  | A    | 76   | 74   | Tmtc | →   |     |     |      |
|          |                      | B    | 65   | 66   | Tmtc | →   |     |     |      |
|          | $.5 \times 10^{-9}$  | A    | 10   | 12   | 10   | 9   | 0   | 0   | 0    |
|          |                      | B    | 5    | 6    | bad  | 5   | 0   | 0   | Tmtc |
|          |                      | C    | 4    | 6    | 10   | 10  | 2   | 0   | 2    |
|          | $.5 \times 10^{-10}$ | A    | 2    | 0    | 2    | 3   | 0   | 0   | 0    |
|          |                      | B    | 0    | 0    | 0    | 0   | 0   | 0   | 0    |
|          |                      | C    | 1    | 0    | 1    | 0   | 0   | 0   | 0    |

|                       |                      |   |    |    |    |    |    |         |      |
|-----------------------|----------------------|---|----|----|----|----|----|---------|------|
| ② dl 707<br>(4/23/97) | $.5 \times 10^{-8}$  | A | 10 | 37 | 13 | 30 | 31 | 10      | Tmtc |
|                       |                      | B | 6  | 37 | 15 | 17 | 26 | 11      | Tmtc |
|                       | $.5 \times 10^{-9}$  | A | 3  | 3  | 2  | 2  | 2  | 5       | 0    |
|                       |                      | B | 0  | 6  | 0  | 2  | 5  | 3 (bad) | bad  |
|                       |                      | C | 0  | 2  | 0  | 0  | 12 | 9       | 2    |
|                       | $.5 \times 10^{-10}$ | A | 0  | 0  | 0  | 0  | 1  | 0       | 0    |
|                       |                      | B | 0  | 0  | 0  | 0  | 0  | 0       | 0    |
|                       |                      | C | 0  | 0  | 3  | 0  | 1  | 0       | 0    |

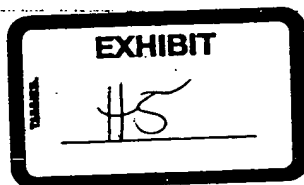
|                       |                      |   |    |    |    |    |      |    |   |
|-----------------------|----------------------|---|----|----|----|----|------|----|---|
| ③ dl 707<br>(3/15/97) | $.5 \times 10^{-8}$  | A | 16 | 41 | 14 | 68 | Tmtc | 19 | → |
|                       |                      | B | 44 | 51 | 15 | 47 | Tmtc | 28 | → |
|                       | $.5 \times 10^{-9}$  | A | 2  | 2  | 2  | 8  | 13   | 3  | 0 |
|                       |                      | B | 1  | 8  | 5  | 2  | 8    | 5  | 2 |
|                       |                      | C | 0  | 2  | 2  | 9  | 14   | 6  | 2 |
|                       | $.5 \times 10^{-10}$ | A | 0  | 0  | 0  | 0  | 1    | 0  | 0 |
|                       |                      | B | 0  | 0  | 0  | 0  | 0    | 1  | 0 |
|                       |                      | C | 0  | 0  | 0  | 0  | 0    | 3  | 0 |

EXHIBIT

44

# 970520 Plaque Assays

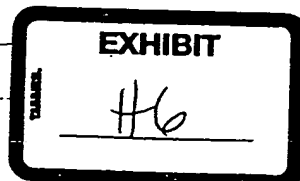
| Virus Dilution            | Dish                 | 5/26          | 5/28           | 5/30 | 6/2  | 6/4 | 6/6 | 6/8  |
|---------------------------|----------------------|---------------|----------------|------|------|-----|-----|------|
| ④ PmE $.5 \times 10^{-8}$ | <del>A</del>         | 42            | 80             |      |      |     |     |      |
|                           | <del>B</del>         | <del>37</del> | <del>102</del> | 74   | TmTC |     |     |      |
|                           | <del>C</del>         | <del>7</del>  | <del>17</del>  | 78   | TmTC |     |     |      |
|                           | A                    | 1             | 8              | 16   | 12   | 4   | 0   | TmTC |
|                           | B                    | 7             | 17             | 11   | 14   | 1   | 0   | TmTC |
|                           | C                    | 7             | 15             | 20   | 7    | 11  | 0   | TmTC |
|                           | $.5 \times 10^{-10}$ | A             | 0              | 0    | 4    | 1   | 2   | 1    |
|                           |                      | B             | 0              | 0    | 3    | 0   | 1   | 0    |
|                           |                      | C             | 0              | 2    | 2    | 2   | 0   | 0    |
|                           |                      |               |                |      |      |     |     |      |
| ⑤ KP $.5 \times 10^{-8}$  | A                    | TmTC          |                |      |      |     |     |      |
|                           | B                    | TmTC          |                |      |      |     |     |      |
|                           | $.5 \times 10^{-9}$  | A             | 20             | 8    | 8    | 3   | 5   | 0    |
|                           |                      | B             | 37             | 7    | 2    | 1   | 2   | 0    |
|                           |                      | C             | 24             | 11   | 11   | 1   | 0   | 0    |
|                           | $.5 \times 10^{-10}$ | A             | 2              | 0    | 1    | 1   | 1   | 2    |
|                           |                      | B             | 0              | 1    | 0    | 0   | 0   | 1    |
|                           |                      | C             | 5              | 0    | 0    | 2   | 1   | 2    |
|                           |                      |               |                |      |      |     |     |      |



Cumulative Counts

970(5/20) Plaque Assays

| Virus                | Dilution             | Dish | 5/26 | 5/28 | 5/30 | 6/2 | 6/4  | 6/6 | 6/8  |
|----------------------|----------------------|------|------|------|------|-----|------|-----|------|
| ① dl751              | $.5 \times 10^{-8}$  | A    | 76   | 150  | TMTc |     |      |     | →    |
|                      |                      | B    | 65   | 131  | TMTc |     |      |     | →    |
|                      | $.5 \times 10^{-9}$  | A    | 10   | 22   | 32   | 41  | 41   | 41  |      |
|                      |                      | B    | 5    | 11   | 11   | 16  | 16   | 16  |      |
|                      |                      | C    | 4    | 10   | 20   | 30  | 32   | 32  | 34   |
|                      | $.5 \times 10^{-10}$ | A    | 2    | 2    | 4    | 7   | 7    | 7   |      |
|                      |                      | B    | 0    | 0    | 0    | 0   | 0    | 0   |      |
|                      |                      | C    | 1    | 1    | 2    | 2   | 2    | 2   |      |
|                      |                      |      | 22   | 46   | 69   | 96  | 98   | 98  | 100  |
| ② dl707<br>(4/23/97) | $.5 \times 10^{-8}$  | A    | 10   | 47   | 60   | 90  | 121  | 131 | TMTc |
|                      |                      | B    | 6    | 43   | 58   | 75  | 101  | 112 | TMTc |
|                      | $.5 \times 10^{-9}$  | A    | 3    | 6    | 9    | 11  | 13   | 18  | 18   |
|                      |                      | B    | 0    | 6    | 6    | 9   | 14   | 17  | 17   |
|                      |                      | C    | 0    | 2    | 2    | 2   | 14   | 23  | 25   |
|                      | $.5 \times 10^{-10}$ | A    | 0    | 0    | 0    | 0   | 1    | 1   | 1    |
|                      |                      | B    | 0    | 0    | 0    | 0   | 0    | 0   | 0    |
|                      |                      | C    | 0    | 0    | 3    | 3   | 4    | 4   | 4    |
|                      |                      |      | 3    | 14   | 20   | 25  | 46   | 63  | 65   |
| ③ dl707<br>(3/15/97) | $.5 \times 10^{-8}$  | A    | 16   | 57   | 71   | 139 | TMTc |     | →    |
|                      |                      | B    | 44   | 95   | 110  | 157 | TMTc |     | →    |
|                      | $.5 \times 10^{-9}$  | A    | 2    | 4    | 6    | 14  | 27   | 30  | 30   |
|                      |                      | B    | 1    | 9    | 14   | 16  | 24   | 29  | 31   |
|                      |                      | C    | 0    | 2    | 4    | 13  | 27   | 33  | 35   |
|                      | $.5 \times 10^{-10}$ | A    | 0    | 0    | 0    | 0   | 1    | 1   | 1    |
|                      |                      | B    | 0    | 0    | 0    | 0   | 0    | 1   | 1    |
|                      |                      | C    | 0    | 0    | 0    | 0   | 0    | 3   | 3    |
|                      |                      |      | 3    | 15   | 24   | 43  | 79   | 97  | 101  |



# 970520 Plaque Assays

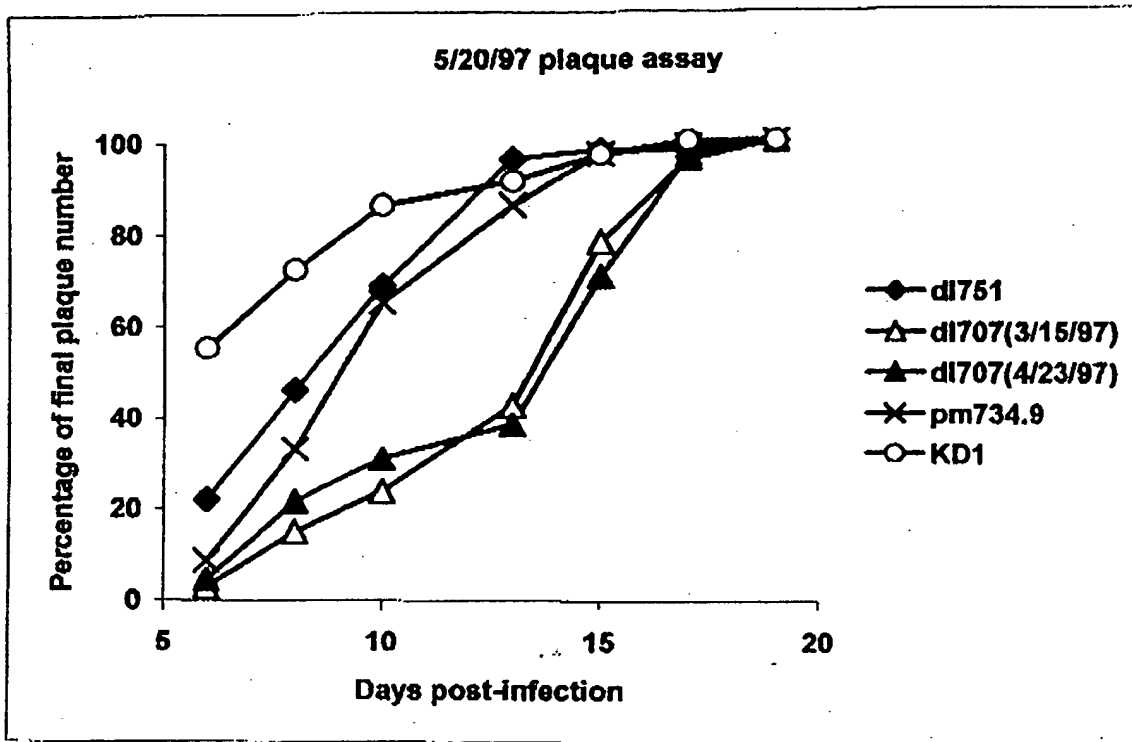
| Virus Dilution            | Dish                   | 5/26 | 5/28 | 5/30 | 6/2  | 6/4 | 6/6 | 6/8  |
|---------------------------|------------------------|------|------|------|------|-----|-----|------|
| ④ PHE $.5 \times 10^{-8}$ | <del>A</del>           |      |      |      |      |     |     |      |
|                           | A                      | 42   | 122  | 196  | TMTc |     |     | →    |
|                           | B                      | 37   | 139  | 217  | TMTc |     |     | →    |
|                           | $.5 \times 10^{-9}$ A  | 1    | 9    | 25   | 37   | 41  | 41  | TMTc |
|                           |                        | B    | 7    | 24   | 35   | 49  | 50  | TMTc |
|                           |                        | C    | 7    | 22   | 42   | 49  | 60  | TMTc |
|                           | $.5 \times 10^{-10}$ A | 0    | 0    | 4    | 5    | 7   | 8   | 10   |
|                           |                        | B    | 0    | 0    | 3    | 3   | 4   | 4    |
|                           |                        | C    | 0    | 2    | 4    | 6   | 8   | 8    |
|                           |                        | 15   | 57   | 113  | 149  | 168 | 171 | 173  |
| ⑤ KD $.5 \times 10^{-8}$  | A                      | TMTc |      |      |      |     |     | →    |
|                           | B                      | TMTc |      |      |      |     |     | →    |
|                           | $.5 \times 10^{-9}$ A  | 20   | 28   | 36   | 39   | 44  | 44  | 44   |
|                           |                        | B    | 37   | 44   | 46   | 47  | 49  | 49   |
|                           |                        | C    | 24   | 35   | 46   | 47  | 47  | 47   |
|                           | $.5 \times 10^{-10}$ A | 2    | 2    | 3    | 4    | 5   | 7   | 7    |
|                           |                        | B    | 0    | 1    | 1    | 1   | 2   | 2    |
|                           |                        | C    | 5    | 5    | 5    | 7   | 8   | 10   |
|                           |                        | 88   | 115  | 137  | 145  | 154 | 159 | 159  |

EXHIBIT

#7

970520 plaque assay\*

|                | 6    | 8    | 10   | 13   | 15   | 17   | 19  |
|----------------|------|------|------|------|------|------|-----|
| dl751          | 22   | 46   | 69   | 96   | 98   | 98   | 100 |
| dl707(3/15/97) | 3    | 14.9 | 23.8 | 42.6 | 78.2 | 96   | 100 |
| dl707(4/23/97) | 4.6  | 21.5 | 30.8 | 38.5 | 70.8 | 96.9 | 100 |
| pm734.9        | 8.7  | 32.9 | 65.3 | 86.1 | 97.1 | 98.8 | 100 |
| KD1            | 55.3 | 72.3 | 86.2 | 91.2 | 96.9 | 100  | 100 |



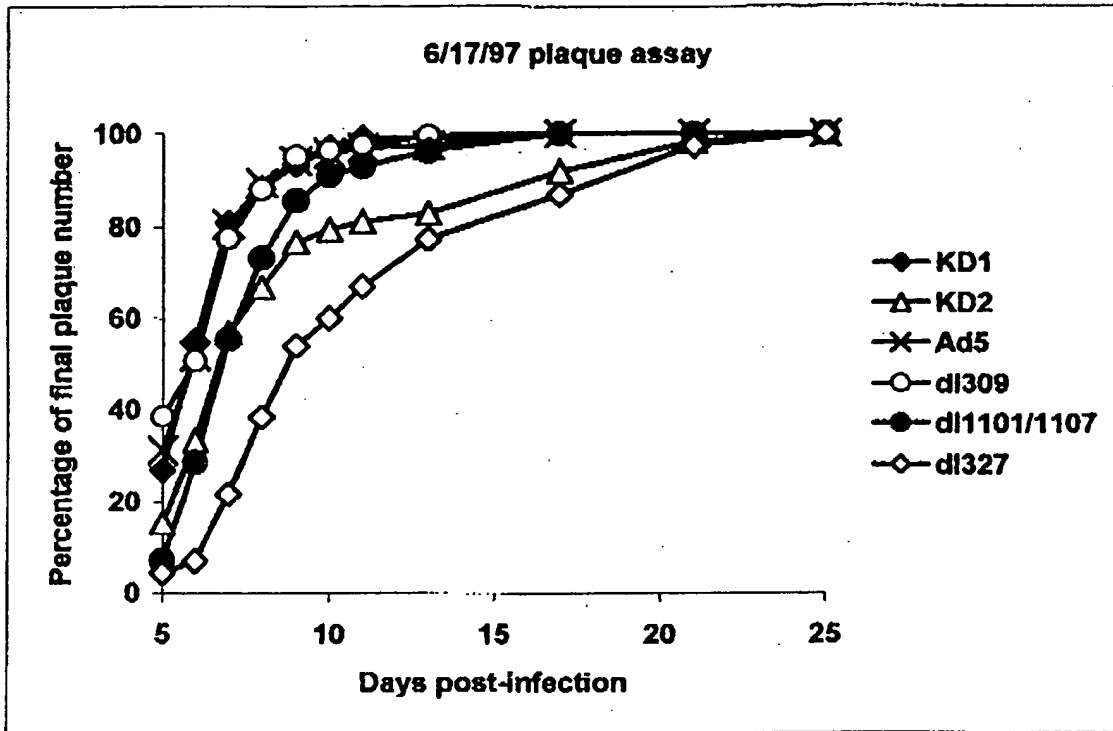
\*numbers at the top of the page are the data points represented in the graph

EXHIBIT

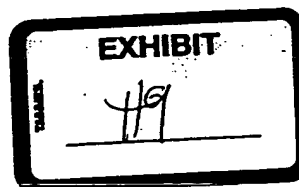
48

970520 plaque assay\*

|             | 5    | 6    | 7    | 8    | 9    | 10   | 11   | 13   | 17   | 21   | 25  |
|-------------|------|------|------|------|------|------|------|------|------|------|-----|
| KD1         | 27   | 55.2 | 80.9 | 88.4 | 93.4 | 97.1 | 99.2 | 99.2 | 100  | 100  | 100 |
| KD2         | 15.3 | 33.3 | 56.8 | 66.7 | 76.6 | 79.3 | 81.1 | 82.9 | 91.9 | 98.2 | 100 |
| Ad5         | 31.2 | 51.9 | 80.6 | 89.5 | 94.1 | 95.8 | 97   | 97.5 | 100  | 100  | 100 |
| dl309       | 38.5 | 50.8 | 77.5 | 88.1 | 95.1 | 96.3 | 97.5 | 99.6 | 100  | 100  | 100 |
| dl1101/1107 | 7.1  | 28.6 | 55.4 | 73.2 | 85.7 | 91.1 | 92.9 | 96.4 | 100  | 100  | 100 |
| dl327       | 4.3  | 7    | 21.7 | 38.3 | 53.9 | 60   | 67   | 77.4 | 87   | 97.4 | 100 |



\*numbers at the top of the page are the data points represented in the graph





(7/26) Blue

(5/28) Green

#6

970526

(5/20) Plaque Assays

| <u>Virus</u>       | <u>Dilution</u>   | <u>A</u>                       | <u>B</u>                         | <u>C</u>                         | <u>Comments</u>   |
|--------------------|---|--------------------------------|----------------------------------|----------------------------------|---|
| ① dl751            | $0.5 \times 10^{-8}$<br>$.5 \times 10^{-9}$<br>$.5 \times 10^{-10}$ | 76+74<br>10+12<br>2+0          | 65+66<br>5+6<br>0 <sup>0</sup>   | <del>4+6</del><br>1+0            | quite large +<br>very distinct  |
| ② dl 707<br>(1/23) | $.5 \times 10^{-8}$<br>$.5 \times 10^{-9}$<br>$.5 \times 10^{-10}$  | 10+37<br>3+3<br>0 <sup>0</sup> | 6+37<br>0+6<br>0 <sup>0</sup>    | <del>0+2</del><br>0 <sup>0</sup> | small, flat +<br>very indistinct  |
| ③ dl 707<br>(3/5)  | $.5 \times 10^{-8}$<br>$.5 \times 10^{-9}$<br>$.5 \times 10^{-10}$  | 16+41<br>2+2<br>0 <sup>0</sup> | 44+51<br>1+8<br>0 <sup>0</sup>   | <del>0+2</del><br>0 <sup>0</sup> | flat + <u>indistinct</u>  |
| ④ PME              | $.5 \times 10^{-8}$<br>$.5 \times 10^{-9}$<br>$.5 \times 10^{-10}$  | 46+10<br>1+8<br>0 <sup>0</sup> | 37+102<br>7+17<br>0 <sup>0</sup> | <del>7+15</del><br>0+2           | fairly large +<br>quite distinct  |
| ⑤ KP               | $.5 \times 10^{-8}$<br>$.5 \times 10^{-9}$<br>$.5 \times 10^{-10}$  | TNTC<br>20+8<br>2+0            | TNTC<br>37+7<br>0+1              | <del>24+11</del><br>5+0          | small to large<br>all distinct; "cleared"<br>center in many plaques<br>(all cells lysed?) |
| ⑥ mock             | —   | 0                              | 0                                | <del>X</del>                     |   |

AT Counts + descriptions  
of Shari O'Briens  
(5/20) Plaque Assay  
Comments most likely done on  
15/1/1

EXHIBIT

#10

## **EXHIBIT I**

Analysing the infectivity of the viruses 1101/1107 and d1309  
on Growth arrested and growing cells.

cell line = HEL 299 Human Lung embryonic.

Grown as in ATCC literature

T. 75 flask 1 from Karl,

split to 1:30 = 35 mm dish.  
1:60 = 35 mm dish. (how many)  
(for growing media)

35 mm dish.

uninfected

1101/1107

d1309

1) 35 mm + cover glass

how many days? 2 2  
6 days

Growth  
arrested

Growing

6

6

6

6

(28)

2) 35 mm + coverslip =

how many days? 6 days

titrate each point 1-6 days or

EXHIBIT

II

|      |         |      |       |      |        |       |       |     |
|------|---------|------|-------|------|--------|-------|-------|-----|
| PHOC | IN U.S. | A WE | JSHIN | Y II | DING I | A.W.D | B 879 | I 3 |
|------|---------|------|-------|------|--------|-------|-------|-----|

|      |       |     |                |
|------|-------|-----|----------------|
| 1:30 | Split | for | growth studies |
|------|-------|-----|----------------|

Gmina

741

12

2 (1)



1, 2, 3

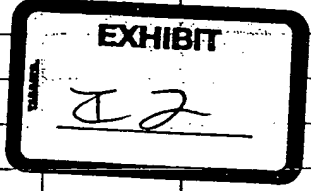
Cover Slip

day 3

for Ad fiber IF.

10

22

$$d(1101)/1107 = p_{\text{free}} \quad \checkmark$$


HEL 299

↓ 7.75

35 mm dishes

1:30,

1:60

↓

MEM; 0.1mM Non essential a.a.s; 1mM Sodium-pyruvate + 0.1% lactalbumin hydrolysate + 10% FBS

↓ 1:60

Split, count the cells + infect E

d1 110/11071, d1 309, 100 moi

↓ 1:30

Split, allow to become confluent, then growth arrest by low serum, (3 day) then infect E d1 1101/d1 1107

0.2%

↓

EXHIBIT

73

viral infection d1 1101/1107

- count one growing MEL 299 cells.  
- 100 pfu -

- Make 200 ml EMEM + NaPyruvate, NEAA + Lactalbumin Lychee  
100x 100x 100x

$$\frac{116}{31} = 3.74 \times 2 = 7.48 \quad \boxed{294,000 / \text{ml}}$$

d1 309 - 950111 - pfu  $2.15 \times 10^{11}$  Location 5-7

d1 1101/1107 - from Karl - pfu  $1.95 \times 10^{11}$

from 35 mm - MEL 299 growing cells - control -  $294,000 / \text{ml}$

$$\begin{aligned} 100 \text{ pfu } d1 \text{ 309} &= \frac{100 \times 294,000}{2.15 \times 10^{11}} \\ &= \frac{294}{2.15 \times 10^6} = \frac{2.94}{2.15 \times 10^4} = 0.000136 \end{aligned}$$

= Virus is too concentrated.  
Dilute to  $10^9$   
10ml dilute to 1ml in medium (no serum) gives  
-  $2.15 \times 10^9$

EXHIBIT  
24

$$= \frac{100 \times 294}{2.15 \times 10^9} = \frac{294}{2.15 \times 10^7} = 1.37 \times 10^{-5}$$

$$\begin{array}{lcl}
 \text{dl 1101 / dl 1107 } 100 \text{ pfu} & = & \frac{100 \times 294,000}{1.95 \times 10^4} = \frac{294}{1.95 \times 10^4} \\
 \text{dilute the virus to } 10^4 & & \\
 \text{in } 10 \mu\text{l to } 1 \text{ ml} & & \\
 & = & \boxed{15 \mu\text{l}}
 \end{array}$$

12/16/96

Growing cells

2 x 35 mm dish dl 309, 100 pfu

2 x 35 mm dish 11101/1107, 100 pfu

6 x 35 mm on coverslip dl 1101/1107 100 pfu

EXHIBIT  
75

Growth arrested HEL 293 = infectious dil<sup>1</sup> 308, 2/11/07

35 mm dish Medium + 0.29 FCS - 3 days

cell counts = 341,000

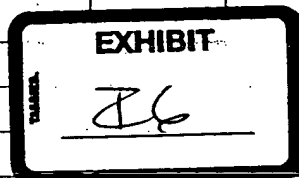
$$\text{dil } 308 = 100 \text{ pfa} = \frac{341 \times 10^3 \times 100}{2.15 \times 10^4} = 15.8 \text{ /ml}$$

$$\text{dil } 1101 = 100 \text{ pfa} = \frac{341 \times 10^3 \times 100}{1.95 \times 10^4} = 17.4 \text{ /ml}$$

Virus 10 µl to 1ml → 10?

↓  
then 37°C / 1hr

↓  
Medium + 0.29 FCS.

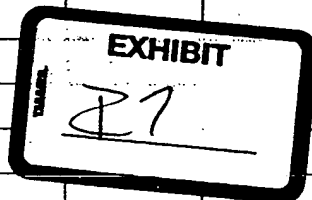




Fixing the HEL299 cells  $\bar{c}$  Methanol + DAPI ( $1^{mg}/ml$ )  
(1  $\mu$ l to 1ml)  
Methanol)

1. Wash the cells 2x PBS w/o
2. Fix  $\bar{c}$  Methanol (-20°C) + DAPI ~ 8 min (-20°C)
3. Wash in Methanol w/o DAPI
4. Wash  $\bar{c}$  1x PBS, store at 4°C

|    |                  |          |        |  |
|----|------------------|----------|--------|--|
| ✓  | 1 day            | 12/17    |        |  |
| ✓  | 2 day            | 12/18    |        |  |
| ✓  | 3 day            | 12/19/96 | →      | $\bar{c}$ d1309 started showing CPE              |
| ✓  | 4 day            | 12/20 -  | NO CPE |  |
|    | 5 "              | 12/21 -  | NO CPE | - d1309 complete CPE                             |
|    | 6 "              |          |        |  |
|    | 7 day            | 12/23 -  | NO CPE | ) all d1309 & cells infect rounded & came off    |
|    | 8 day            | 12/24 -  | NO CPE |  |
|    | 9 "              | 12/25    |        |  |
| →  | 10 day           | 12/26 -  | CPE?!  | cells look different re d1101/1107               |
| F  | 11               | 12/27    |        |  |
| S  | 12               | 12/28    |        |  |
| Sm | 13               | 12/29    | →      | - CPE? starts on 12 <sup>th</sup> day d1101/1107 |
| ✓  | M                | 14       | 12/30  | - CPE? spreads - d1101/1107                      |
|    | 15               | 12/31    |        | " "  |
|    | 16               | 1/1      |        |  |
| ✓  | 17               | 2/1      | →      | Take photographs -                               |
|    | 18               | 3/1      | →      |  |
|    | 19               | 4/1      | →      |  |
|    | 20               | 5/1      | →      | almost all the cells died - d1101/               |
|    | 25 <sup>th</sup> | 10/1     | →      | Stain - dish, $\bar{c}$ crystal violet           |
|    |                  |          |        | 2 - d1101/1107                                   |
|    |                  |          |        | 2 - d1309  |



THOUGHTS IN U.S.A.

**EXHIBIT**  
T-100  
18

HEL299 cells

Kodak

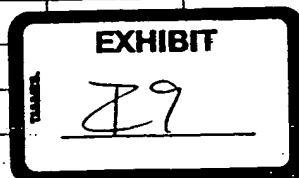
17 days

7

Tmax 100  
black white print

Growing HEL 299

|    |              |              |
|----|--------------|--------------|
| 1  | mock         | N            |
| 2  | "            | +1           |
| 3  | "            | -1           |
| 4  | "            | -1           |
| 5  | "            | N            |
| 6  | "            | +1           |
| 7  | 411101/1107  | N            |
| 8  | "            | +1           |
| 9  | "            | -1           |
| 10 | "            | N            |
| 11 | "            | +1           |
| 12 | "            | -1           |
| 13 | "            | N            |
| 14 | "            | +1           |
| 15 | "            | -1           |
| 16 | "            | N            |
| 17 | "            | +1           |
| 18 | "            | -1           |
| 19 | "            | N            |
| 20 | "            | +1           |
| 21 | "            | -1           |
| 22 | "            | N            |
| 23 | "            | +1           |
| 24 | "            | -1           |
| 25 | "            | N            |
| 26 | "            | +1           |
| 27 | "            | -1           |
| 28 | "            | N            |
| 29 | "            | +1           |
| 30 | "            | -1           |
| 31 | <del>"</del> | <del>N</del> |
| 32 | "            | N            |
| 33 | "            | +1           |
| 34 | "            | -1           |
| 35 | mock         | N            |
| 36 | mock         | +1           |



IF: HEL 299: Growing:

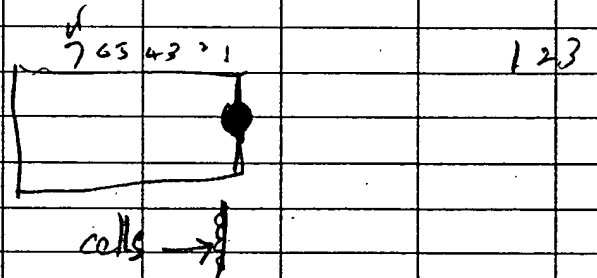
1. ab - Filter, → 1:400  
 DBP → 1:400  
 2. Goat anti mouse Rhodamine 1:50 (not preabsorb)  
 Goat anti Rabbit FITC 1:25

1 = 1<sup>st</sup> layer  
 2 = 2<sup>nd</sup>  
 3 = 3<sup>rd</sup>  
 4 = 4<sup>th</sup>  
 5 = 14<sup>th</sup>  
 6 = 17<sup>th</sup> - little broken  
 7 = CTRL

Al 1101/1107  
 α DBP p 67  
 66420  
 850025  
 4D2-5  
 ascites fluid

FITC = DBP  
 FITC = FITC

1. ab = Ad Fiber dil 1:400  
 = DBP dil 1:400 in 1 tube  
 35 μl / dish → 37°C / 30 min  
 wash: 0.1% BSA w/o, 10 min x 3 times



2. Goat α mouse Rhodamine 1:50  
 Goat α Rabbit FITC 1:25  
 37°C / 15 min  
 8 → 400  
 16 → 400  
 3764  
 64  
 16  
 400

EXHIBIT

10

# Growth Assay

Growth arrested cells.

HE2 299

T-75 → split 15 x 35 mm dish.

# dishes same like growing

↓  
add EMEM + 0.2% FCS

↓  
3 days

↓  
infect  $\bar{e}$  [dl 1101 / 1102]  
pm 975

the control dl 309.

total dish # 27

EXHIBIT

211

# Growth Assay

HEL 299 T-25 Growing:

↓  
Split to 35 x 35 mm dish

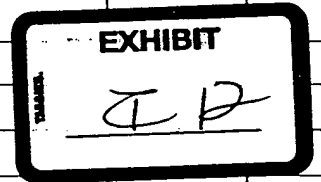
① 1 + 10 dish days (1, 4, 6, 8, 10, 12, 14, 16, 18, 20)  
d1 1101/1107

② 1 + 10 dish Same days - pm 975

↓  
input 100 pfu, freeze the dishes  
as days shown.

③ 4 dishes input & d1 309 for true infection  
Freeze 1, 4, 6 days.

total dishes 27



1/14/98

|           |              |                           |
|-----------|--------------|---------------------------|
| Infection | d1 1101/1107 | pfu $1.95 \times 10^{11}$ |
|           | d1 309       | $2.15 \times 10^{11}$     |
|           | pm 975       | $4.06 \times 10^{10}$     |

Growing HEL 299 cells counted: 212,000/ml

① 100 pfu

|   |   |  |   |
|---|---|--|---|
| For d1 1101/1107  | = | $\frac{100 \times 212,000}{1.95 \times 10^{11}}$ | $= \frac{2.12}{1.95 \times 10^2} = 10.86$ |
| Dilute the virus to $10^9$<br>10 ml to 1 ml medium No serum |   |  |   |

|  |   |   |   |
|--|---|---|---|
| ② For pm 975   | = | $\frac{100 \times 2.12 \times 10^5}{4.06 \times 10^{10}}$ | $= \frac{2.12}{4.06 \times 10^2} = 52.16$ |
| Dilute the viruses to $10^9$<br>50 ml to 500 ml serum free media |   |   |   |

# Growth Curve

## Growing cells

### Summary

③ the CTRL

virus dil 3090  
dilute the virus to  $10^{-9}$

10  $\mu$ l to 1ml serum free  
media

$$= 10^6 \times 2.12 \times 10^6$$

$$2.15 \times 10^7 \times 2$$

$$= \frac{2.12}{2.15 \times 10^2} = 9.8 \mu$$

1. wash the cells in PBS 4x
2. infect in serum free media 37°C / 1 hr
3. After 1 hr, remove the media, wash in media
4. Add MEM + 10% FCS.

Freeze the cells after

|         |        |   |   |
|---------|--------|---|---|
| 1-17-97 | 1 day  | G1  | 1/15/97 ✓   |
|         | 3 days | G2  | dil 300, PM975 shows CPE<br>NO CPE in dil 1101/1107 |
| 1-19/97 | 5      | G3  | N 60% CPE in PM975<br>NO CPE in dil 1101            |
|         | 6      | G4  | PM975 ✓ NO CPE in<br>1101                           |
|         | 7      | G5  | PM975 ~100% ✓                                       |
|         |        | G3  | dil 1101/1107 ~CPE? - NO                            |
| 10      | 10     | PM975 dil 1101/1107 (NO CPE yet)          |   |
|         |        | dil 300 (Photos)                          |   |
|         | 15     | dil 1101/1101 (morphology change<br>CPE?) |   |
|         | 24     | dil 1101/ G5                              |   |

EXHIBIT

13

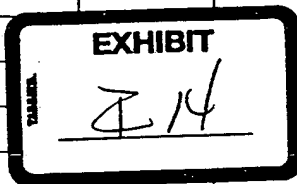
# Growing II

7

|     |    |          |                    |              |               |              |             |                     |         |  |
|-----|----|----------|--------------------|--------------|---------------|--------------|-------------|---------------------|---------|--|
|     |    | infected | 1/31/97            |              |               |              |             |                     |         |  |
|     |    |          | 100<br>d1309       | 500 p6       | 100<br>pm975  | 500          | 1<br>d11101 | 100<br>1107         | 500     |  |
| Day | 2  |          | NO CPE             | "            | NO CPE        | "            | NO CPE      | "                   |         |  |
| Day | 2  |          | Few cells<br>found | Few<br>cells | Few<br>cells  | Few<br>cells |             | NO CPE              | "       |  |
| Day | 3  |          | 10% CPE            | -20% CPE     | 20%           | 50% CPE      |             | NO CPE              |         |  |
| Day | 6  |          | 100% CPE in        |              | pm 975; d1309 |              |             |                     |         |  |
|     |    |          | NO CPE in          |              | d11101/1107   |              |             |                     |         |  |
| Day | 14 |          | —                  | —            |               |              |             | 500 p6 - CPE        | starts. |  |
| Day | 7  |          | —                  | —            |               |              |             | 100 p6 - CPE starts |         |  |
|     |    |          |                    |              |               |              |             | 500 p6 - CPE ++     |         |  |

EXHIBIT

214





# Growth arrested MEL288

22/6/1

G1

Days

3

pm975 - -70 C

5

pm975, dl 1101/1107, dl 309 (photos)

7

pm975 -70 C (photos)

10

pm975; dl 1101/1107 (NO LPE)

(GAT)

(GAT)

14

pm975

(GAT3)

15

-pm 975 NO CPE

50% cells from control & pm975. dl 1101/1107  
the same exp. NO contamination.  
Maybe due to not changing medium?

19

pm975 (GAT)

EXHIBIT

15

Growing cells  
on coverslips  
for IF & CPE

HEL 299 cells

counted 1 dish = 188,000 cells = 1.35 x 10<sup>6</sup>

① virus pm 925 pfu 4.06 x 10<sup>10</sup>

$$\text{for } 100 \text{ pfu} = \frac{100 \times 1.88 \times 10^4}{4.06 \times 10^{10}} = \frac{1.88}{4.06 \times 10^2}$$

dilute the virus to 10<sup>9</sup> titer.

50 µl to 500 µl serum free media

= 4.6 µl

② virus dl 101/1107

$$= \frac{100 \times 1.88 \times 10^4}{1.95 \times 10^9} = \frac{1.88}{1.95 \times 10^2}$$

dilute the virus to 10<sup>9</sup>

10 µl to 1ml medium.

= 9.6 µl

③ +ve control dl 309

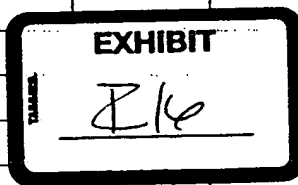
$$= \frac{100 \times 1.88 \times 10^4}{2.15 \times 10^9} = \frac{1.88}{2.15 \times 10^2}$$

dilute the virus to 10<sup>9</sup>

10 µl to 1ml medium

= 8.7 µl

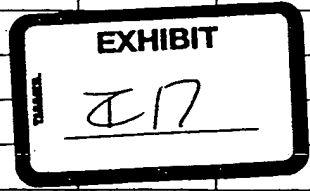
10<sup>4</sup> 10



# Growth arrested HEL 299<sup>P12</sup> Cells P<sup>12</sup>

24 days

|    |                        |        |
|----|------------------------|--------|
| 0  | HEL 299 (Mock)         | = 0    |
| 1  | "                      | +1     |
| 2  | "                      | -1     |
| 3  | "                      | 0      |
| 4  | "                      | +1     |
| 5  | "                      | -1     |
| 6  | HEL 299 + dl 1101/1107 | = 0    |
| 7  | "                      | +1     |
| 8  | "                      | -1     |
| 9  | ↑                      |        |
| 10 |                        |        |
| 11 |                        |        |
| 12 |                        |        |
| 13 |                        |        |
| 14 |                        |        |
| 15 |                        |        |
| 16 |                        |        |
| 17 |                        |        |
| 18 |                        |        |
| 19 |                        |        |
| 20 |                        |        |
|    | Growing cells          | 3 days |
| 21 | HEL 299 Mock           | = 0    |
| 22 | "                      | +1     |
| 23 | "                      | -1     |
| 24 | + PM 175               | 2      |
| 25 | "                      |        |
| 26 | "                      |        |
| 27 | dl 1101/1107           |        |
| 28 | "                      |        |
| 29 | "                      |        |
| 30 | dl 309                 |        |
| 31 | "                      |        |
| 32 | "                      |        |
| 33 | PM 975                 |        |
| 34 | ↓                      |        |
| 35 |                        |        |
| 36 |                        |        |
| 37 |                        |        |



2/2/87

UTAHIN NATION HEL 211

infected E d1101/1107

24th day

TMY 400 B+W

100

1-10

25

1-80

REA

MP

Y PUBL

DUICK

1-1

SAFHEI

|    |                      |     |
|----|----------------------|-----|
| 1  | HEL 299 (mole)       | = 0 |
| 2  | "                    | +1  |
| 3  | "                    | -1  |
| 4  | "                    | 0   |
| 5  | "                    | +1  |
| 6  | "                    | -1  |
| 7  | HEL 299 + d1101/1107 | = 0 |
| 8  | "                    | +1  |
| 9  | "                    | -1  |
| 10 | "                    | 0   |
| 11 | "                    | +1  |
| 12 | "                    | -1  |
| 13 | "                    | 0   |
| 14 | "                    | +1  |
| 15 | "                    | -1  |
| 16 | "                    | 0   |
| 17 | "                    | +1  |
| 18 | "                    | -1  |
| 19 | "                    | 0   |
| 20 | "                    | +1  |
| 21 | "                    | -1  |

print reads 0=1

Same

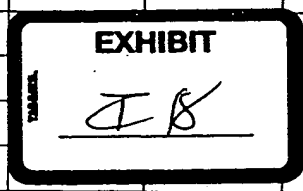
Growing cells 3rd day

2-23

Growing 21, 22, 23 mole

|    |              |     |
|----|--------------|-----|
| 21 | HEL 299 MOLE | = 0 |
| 22 | "            | +1  |
| 23 | "            | -1  |
| 24 | "            | 0   |
| 25 | + PM 975     | 0   |
| 26 | "            | +1  |
| 27 | "            | -1  |
| 28 | d1101        | 0   |
| 29 | "            | +1  |
| 30 | "            | -1  |
| 31 | d1309        | 0   |
| 32 | "            | +1  |
| 33 | "            | -1  |
| 34 | PM 975       | = 0 |
| 35 | "            | +1  |
| 36 | "            | -1  |
| 37 | "            | 0   |

Same



# Growth arrested HEL 299 cells

## - Growth Curve:-

Count the cells: 347,000 cells

① 100 pfu =  
for dilution 10<sup>4</sup>

$$= \frac{100 \times 347,000}{1.95 \times 10^4} = \frac{347}{1.95 \times 10^4} = 17.2$$

dilute the virus to 10<sup>4</sup>  
1 ml to 1 ml

② 100 pfu  
for pm 975

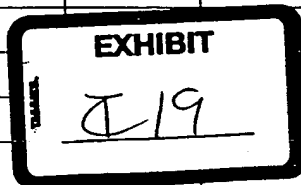
$$= \frac{100 \times 347,000}{4.06 \times 10^4} = \frac{347}{4.06 \times 10^4} = 8.52$$

dilute the virus to 10<sup>4</sup>  
50 ml to 500 ml

③ the count is 309  
dil to 10<sup>4</sup>  
10 ml to 1 ml

$$= \frac{100 \times 347,000}{2.15 \times 10^4} = \frac{347}{2.15 \times 10^4} = 16.1$$

↓  
work  
infant  
EMC 0.29 FCS



P111

photographs:

Tmax 170 B/w

Growth arrested HEL299, 3 days

C

30

2

100

ADING

BLISH

IN-WI

ED IN

111

|    |               |     |
|----|---------------|-----|
| 1  | HEL299 - MOLA | = 0 |
| 2  | "             | +1  |
| 3  | "             | -1  |
| 4  | "             | 0   |
| 5  | "             | +1  |
| 6  | "             | -1  |
| 7  | + pm 975      | 0   |
| 8  | "             | +1  |
| 9  | "             | -1  |
| 10 | "             | 0   |
| 11 | "             | +1  |
| 12 | "             | -1  |
| 13 | "             | 0   |
| 14 | "             | +1  |
| 15 | "             | -1  |
| 16 | "             | 0   |
| 17 | "             | +1  |
| 18 | "             | -1  |

pm 975, 3<sup>rd</sup> day, 6A

nucleus appears darker than control

✓ KRT -

EXHIBIT

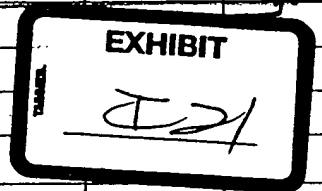
420

P/T  
in photos

Growing 9<sup>th</sup> day

Tmax 100 B/W Prints

|                                     |                |              |                      |
|-------------------------------------|----------------|--------------|----------------------|
| 1                                   | HEL 299 (Mock) | 2 NCO        |                      |
| 2                                   | "              | +1           |                      |
| 3                                   | "              | -1           |                      |
| 4                                   | "              | 0            |                      |
| 5                                   | "              | +1           |                      |
| 6                                   | "              | -1           |                      |
| 7                                   | d1 309         | 0            |                      |
| 8                                   | "              | +            |                      |
| 9                                   | "              | -1           | CPE ✓                |
| 10                                  | "              | 0            |                      |
| 11                                  | "              | +1           |                      |
| 12                                  | "              | -1           |                      |
| 13                                  | pm 975         | 0            |                      |
| 14                                  | "              | +1           |                      |
| 15                                  | "              | -1           | CPE ✓                |
| 16                                  | "              | 0            |                      |
| 17                                  | "              | +1           |                      |
| 18                                  | "              | -1           |                      |
| 19                                  | d1 1101        | 0            |                      |
| 20                                  | "              | +1           | 0 ?                  |
| 21                                  | "              | -1           |                      |
| 22                                  | "              | 0            | 0                    |
| 23                                  | "              | +1           |                      |
| 24                                  | "              | -1           |                      |
| Growth arrested 5 <sup>th</sup> day |                |              |                      |
| 25                                  | HEL 299 Mock   | 0            |                      |
| 26                                  | "              | +1           |                      |
| 27                                  | "              | -1           |                      |
| 28                                  | pm 975         | 0            |                      |
| 29                                  | "              | +1           |                      |
| 30                                  | "              | -1           | CPE - NOT yet        |
| 31                                  | "              | 0            | NO CPE sign          |
| 32                                  | "              | +1           |                      |
| 33                                  | "              | -1           |                      |
| 34                                  | d1 309         | 0            |                      |
| 35                                  | "              | +            | Shows - starting CPE |
| 36                                  | "              | -1           |                      |
| 37                                  | "              | 0 less light |                      |



8/

# Photographs

Carl took 1-18 photos

Growth Arrested HEL 289 9th day

|    |          |    |               |
|----|----------|----|---------------|
| 19 | HEL - LA | 0  | ] NO CPE sign |
| 20 | "        | +1 |               |
| 21 | "        | -1 |               |
| 22 | PM975    | 0  |               |
| 23 | "        | +1 |               |
| 24 | "        | -1 |               |
| 25 | "        | 0  |               |
| 26 | "        | +  | ] NO CPE sign |
| 27 | "        | -1 |               |
| 28 | DL1101   | 0  |               |
| 29 | "        | +1 |               |
| 30 | "        | -1 |               |
| 31 | "        | 0  |               |
| 32 | "        | +  |               |
| 33 | "        | -1 | ] shows CPE,  |
| 34 | DL 309   | 0  |               |
| 35 | "        | +  |               |
| 36 | "        | -1 |               |
| 37 | "        | 0  |               |
| 38 | "        | +1 |               |

EXHIBIT

122



P6

photos:

Tmax 100 Black + white

Growth arrested HEL 299 - 10<sup>th</sup> day.

1 HEL 299, GA, mock - 0

2 " " +1

3 " " -1

4 PM975 - 0

5 " " +1

6 " " -1

7 " " 0

8 " " +1

9 " " -1

10 dl 1101 / 1107 - 0

11 " " +1

12 " " -1

13 " " 0

14 " " +1

15 " " -1

16 dl 309 - 0

17 " " +1

18 " " -1

19 " " 0

20 " " +1

21 " " -1

Growing cells → 15 days

22 HEL 299, mock - 0

23 " " +1

24 " " -1

25 PM975 - 0

26 " " +1

27 " " -1

28 dl 309 - 0

29 " " +1

30 " " -1

31 dl 1101 / 1107 - 0

32 " " +1

33 " " -1

34 dl 1101 / 1107 - 0

ND CPE?

ND CPE.

shows CPE - growing

All the cells rounded & most of them floating (dead cells)

All the cells rounded & floating (dead)

Cells are larger, fewer, some cells have larger nuclei - CPE?

EXHIBIT

223

Q1

photographs 3<sup>rd</sup> day  
GROWING HEL 299 P<sup>17</sup>

|    |         |         |   |    |         |          |
|----|---------|---------|---|----|---------|----------|
| 1  | HEL 299 | 6       | 2 | 0  |         |          |
| 2  | "       |         |   | +1 |         |          |
| 3  | "       |         |   | -1 |         |          |
| 4  | dl 309  | 100 pfn |   | 0  |         |          |
| 5  | "       |         |   | +1 | 100 pfn | ~ 109 W5 |
| 6  | "       |         |   | -1 |         |          |
| 7  | dl 309  | 500 pfn |   | 0  |         |          |
| 8  | "       |         |   | +1 | 500 pfn | ~ 309    |
| 9  | "       |         |   | -1 |         |          |
| 10 | pm 475  | 100 pfn |   | 0  |         |          |
| 11 | "       |         |   | +1 |         |          |
| 12 | "       |         |   | -1 | 100 pfn | ~ 209    |
| 13 | "       |         |   | 0  |         |          |
| 14 | "       |         |   | +1 |         |          |
| 15 | "       |         |   | -1 |         |          |
| 16 | pm 475  | 500 pfn |   | 0  |         |          |
| 17 | "       |         |   | +1 |         |          |
| 18 | "       |         |   | -1 | 500 pfn | ~ 419    |
| 19 | "       |         |   | 0  |         |          |
| 20 | "       |         |   | +1 |         |          |
| 21 | "       |         |   | -1 |         |          |
| 22 | dl 1101 | 100 pfn |   | 0  |         |          |
| 23 | "       |         |   | +1 |         |          |
| 24 | "       |         |   | -1 | 100 pfn | NO CPE   |
| 25 | "       |         |   | 0  |         |          |
| 26 | "       |         |   | +1 |         |          |
| 27 | "       |         |   | -1 |         |          |
| 28 | dl 1101 | 500 pfn |   | 0  |         |          |
| 29 | "       |         |   | +1 |         |          |
| 30 | "       |         |   | -1 | 500 pfn | NO CPE   |
| 31 | "       |         |   | 0  |         |          |
| 32 | "       |         |   | +1 |         |          |
| 33 | "       |         |   | -1 |         |          |
| 34 | HEL 299 | CONTROL |   | 0  |         |          |
| 35 | "       |         |   | +1 |         |          |
| 36 | "       |         |   | -1 |         |          |

EXHIBIT

224



Expt 3

Growth arrested 412 299 cells p<sup>171</sup>

- Growth curve:-

Count the cells: 347,000 cells

① 100 pfr =  $\frac{100 \times 347,000}{1.95 \times 10^4} = \frac{347}{1.95 \times 10^4} = 17.1$

dilute the virus to 10<sup>4</sup>

10 ml to 1 ml

500 pfr = 85 ml

② 100 pfr =  $\frac{100 \times 347,000}{4.06 \times 10^4} = \frac{3.47}{4.06 \times 10^4} = 8.5$

dilute the virus to 10<sup>4</sup>

50 ml to 500 ml

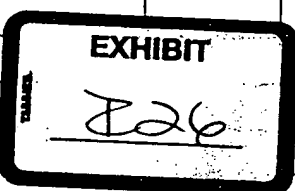
500 pfr = 42.5 ml

③ the count of 1309 =  $\frac{100 \times 3.47 \times 10^4}{2.15 \times 10^4} = \frac{3.47}{2.15 \times 10^2} = 16$

dil to 10<sup>4</sup>

10 ml to 1 ml

500 pfr = 80 ml



↓

wash

infest

EMEM 0.24 FCS

NO CPE

Days 2 =

Days 3 =

3/10/97

d1 1101, pm 975, d1309 NO CPE / 100 pfr  
at 500 pfr, 2 pm 975 & d1309 shows  
beginning of CPE

Day 4 =

Day 5 =

Day 6 =

Day 7 =

2/13/97

NO CPE 1101/1107 (100 d 500 pfr)  
NO CPE PM 975 / 100 pfr  
CPE begins pm 975 500 pfr only  
NO CPE PM 975 100 pfr  
NO CPE d1101/100 & 500 pfr  
NO CPE d1309 100 pfr CPE in d1309 500 pfr

Growth Curve:- Frozen:- days

KEI- 298

pm975, dl1101  
 Growing: 1,3,5,6,7, 1,3,5,6,7,10,15,19  
 GA: 3,5,7,10,14 5,7,10 19

pm975 GA: GA  
 $\checkmark$ GA 3 = 14th day  
 $\checkmark$ GA 4 = 10th  
~~GA 2 = 5th~~  
 $\checkmark$ GA 7 = 19th  
~~GA 1 = 3th~~

Growing  
 $\checkmark$ GA 3 = 3th day 67  
 $\checkmark$ GA 5 = 5th " 64  
 $\checkmark$ GA 6 = 6th " 64  
 $\checkmark$ GA 7 = 7th "

dl 1101/1107 GA  
 $\checkmark$ GA 7 = 19th day  
 $\checkmark$ GA 9 = 10th "  
 $\checkmark$ GA 2 = 5th "  
 $\checkmark$ GA 23 = 23th

Growing  
 G3 = 5th day  
 G24 = 15th  
 G24 = 24th day

2/18/97

pm975: GA 10, 14, 19 day  
 Growing = 3, 5, 6, 17 day  
3 & 7?

EXHIBIT  
 127

# Growth Curve - Plaque assay:

293 cells:-

Growing - pm 975 : days 1, 3, 5, 6, 7

GA - pm 975 - 3, 5, 7, 10

#9

Growing - d11101 - 1, 5, 7, 10, 15

GA - 7, 10

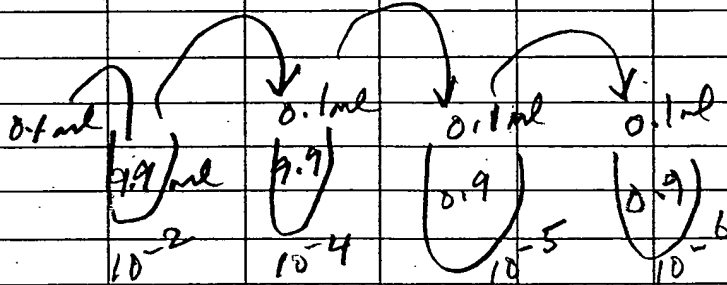
#7

d1309 -

infections = 0.5 ml cells  
0.1 ml  $10^{-2}$   
 $10^{-4}$   
 $10^{-5}$

DO pm 975 first  
# of points Growing = 5  
GA = 4

= 5R = 25 dish  
= 4R = 20 dish  
35



0.5 ml adsorption:  
1 ml  $10^{-2}$  to  $10^{-6}$

0.1 ml cells

Stain on 2/24/97

EXHIBIT

228

dl 1101/1107 Growth Curve:  
plaque assay

dl 1101/1107

GA:

Day

GA7 = 19

GA9 = 10

GA2 = 5

GA = 23

Growing

G3 = 5 hr

G4 = 15 hr def

G24 = 24 hr

GA5 pa925

Dilutions

0.1 ml

9.9 ml

$10^{-2}$

0.1 ml

9.9 ml

$10^{-4}$

0.1

0.9

$10^{-5}$

0.1 ml

0.9

$10^{-6}$



0.5 ml injection:

EXHIBIT

229

# Growth Curve

3/4/97

21101/1007

100 pfu

-Growing

Small plaques

went one more day -

63 = 5<sup>th</sup> day =  $1.4 \times 10^4$   
 15<sup>th</sup> day =  $162 = 406 / 1 \text{ ml} \times 10^2$   
 $= 4.8 \times 10^4$

24<sup>th</sup> day =  $10^{-4}$  66 / 0.5 ml

$132 / 1 \text{ ml}$   
 $= 132 \times 10^4$   
 $= 1.3 \times 10^6 / \text{ml}$

GA

5<sup>th</sup> day =  $1.1 \times 10^3$  pfu/ml (No plaques in ud.)

10<sup>th</sup> day =  $7 \times 10^2 / 0.5 \text{ ml} = 14 \times 10^2$   
 $= 1.4 \times 10^3$

19<sup>th</sup> day = 42 plaques / 0.5 ml (small)  
 $= 84 / 1 \text{ ml} \times 10^2$   
 $= 8.4 \times 10^3$  pfu/ml

23<sup>rd</sup> day =  $28 \times 10^2 / 0.5 \text{ ml}$   
 $= 56 \times 10^2$   
 $= 5.6 \times 10^3$  pfu/ml

EXHIBIT

30



# Growth Curve:

2-25-97

| Growing                   | pm 975:   | 120 pfu             |                             |
|---------------------------|-----------|---------------------|-----------------------------|
| <u>3<sup>rd</sup> day</u> | $10^{-6}$ | <u>plaque</u><br>71 | pfu/ml<br>$7.1 \times 10^7$ |
| 5 <sup>th</sup> day       | $10^{-6}$ | 13                  | $1.3 \times 10^7$           |
| 6 <sup>th</sup> day       | $10^{-6}$ | 11                  | $1.1 \times 10^7$           |
| 7 <sup>th</sup> day       | $10^{-5}$ | 7                   | $7 \times 10^6$             |

| <u>GA</u>            |           |    | pfu/ml            |
|----------------------|-----------|----|-------------------|
| 10 <sup>th</sup> day | $10^{-4}$ | 12 | $1.2 \times 10^5$ |
| 14 <sup>th</sup> day | $10^{-4}$ | 32 | $3.2 \times 10^5$ |
| 19 <sup>th</sup> day | $10^{-4}$ | 18 | $1.8 \times 10^5$ |

GA HEL 29A infected  $\bar{c}$  pm 975 did not CPE, but  
it gave viral yield  $\approx 10^4$

5<sup>th</sup> day is plaque assayed to day 2/25/97  
GA pm 975

EXHIBIT

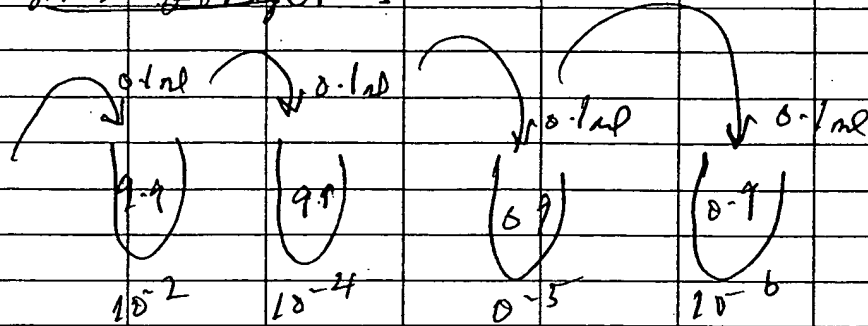
231

# Growth Curve:

3/5/97

pm 975 - growing 1 + 3<sup>rd</sup> log.

~~11/97~~ ~~11/97~~ 5<sup>th</sup>



EXHIBIT

152

# Growth Curve: plaque assay

11/1/75

Growing:

G1 day d1 1101/1107  
G2 (3 day) " "

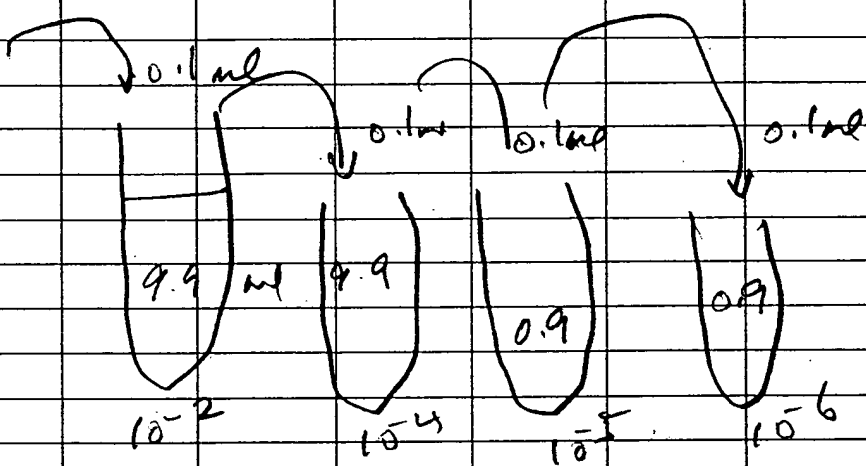
G1 d1 309  
G5 d1 309

G A = G A1 d1 1101/1107

G A1, d1 309  
G A5 - d1 309

7 viruses

Dilutions



0.5 ml

EXHIBIT

233

ST. S. Grand: Amos  
867-8292  
743-10891  
984-2123

# Growth Curve.

3.11.97

Growing:-

Day 1 (G1) <sup>PM 475</sup>

$$= 4 \times 10^{-4}$$

$$= 4 \times 10^4$$

0.5ml

$$= 8 \times 10^4 / \text{ml}$$

6<sup>th</sup> day infection

Day 3

$$= 1.9 \times 10^{-5}$$

$$1 \times 10^{-6}$$

$$= 1.9 \times 10^6$$

wait 2 more days.

↓ 0.5ml

$$3.8 \times 10^6 / \text{ml}$$

very small plaques.  
allow two more days to  
form more plaques.

$$31 \times 10^6 \text{ (counted after 2 days)}$$

$$\downarrow 3.1 \times 10^7 \text{ PFU/ml}$$

EXHIBIT

234

# Growth arrested.

3/31/97

500 pfu

HEL 299 = \* cells 351,000

1) for dil 1101/07  
dilute virus to  $10^7$   
(10  $\mu$ l to 1ml)

$$= \frac{5}{500} \times 351,000 = \frac{5 \times 351}{1.95 \times 10^4} = 90 \text{ ml}$$

$10^7$   
 $10^4$

2) for dil 309  
dilute the virus to  $10^9$   
(10  $\mu$ l to 1ml)

$$= \frac{5}{500} \times 351,000 = \frac{5 \times 351}{2.15 \times 10^4} = 81 \text{ ml}$$

$10^9$   
 $10^4$

↓  
wash  
infect, harvest,  
add EMEM + 0.2% FCS.

Freeze) 1 hour 3/31/97 ✓  
1 day  
6  
8  
15

6  
10  
15  
21

EXHIBIT

735

# CPE Table

|     |  |  |  | Growing (100 pm/cell)  |       |           | HEL 299 - P <sup>17</sup> |
|-----|--|--|--|------------------------|-------|-----------|---------------------------|
|     |  |  |  | d1309                  | pm975 | d1101/107 |                           |
| Day |  |  |  |                        |       |           |                           |
| 1   |  |  |  | -                      | -     | -         |                           |
| 3   |  |  |  | +                      | +     | -         |                           |
| 5   |  |  |  | +++                    | +++   | -         |                           |
| 6   |  |  |  | ++++                   | ++++  | -         |                           |
| 7   |  |  |  |                        |       | -         |                           |
| 10  |  |  |  |                        |       | -         |                           |
| 15  |  |  |  |                        |       | ±         | (cell morphology changes) |
| 24  |  |  |  |                        |       | ++        |                           |
|     |  |  |  | <u>Growth arrested</u> |       |           |                           |
| Day |  |  |  |                        |       |           |                           |
| 1   |  |  |  | -                      | -     | -         |                           |
| 3   |  |  |  | ±                      | -     | -         |                           |
| 5   |  |  |  | +                      | ±     | -         |                           |
| 9   |  |  |  | ++                     | -     | -         |                           |
| 10  |  |  |  | +++                    | -     | -         |                           |
| 19  |  |  |  |                        | +     | -         |                           |
| 24  |  |  |  |                        | +     | -         |                           |

EXHIBIT

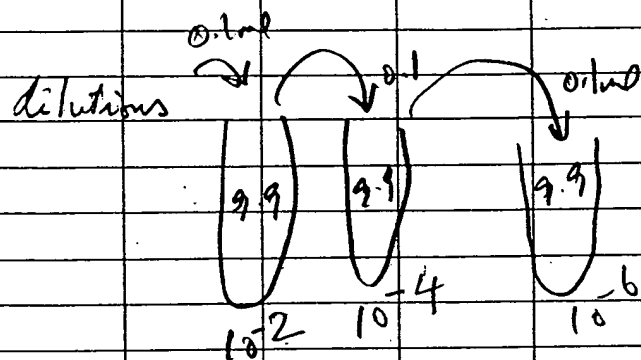
730

Titer: 283 cells d1 309 GA HEL 29

0120111

d1 309 GA:

800 pfu/ml



3h, 1d, 4, 6, 8 days.  
16 & 15

EXHIBIT

237

5167111  
5/15/97  
5/19/97

HEL 299 cells

500 pfu/cell

Growing

d1309 d1101/07

|                   |                   |   |
|-------------------|-------------------|---|
| 3 hrs             | $9 \times 10^3$   | $2 \times 10^4$                               |
| 1 day             | $1 \times 10^4$   | $4.2 \times 10^4$                             |
| 4 "               | $1.6 \times 10^7$ | $1.2 \times 10^5$                             |
| 8 " $\rightarrow$ | $2.3 \times 10^7$ | $1.4 \times 10^5$                             |
| 10 "              | $7 \times 10^6$   | $2.4 \times 10^5 \rightarrow 7.1 \times 10^5$ |
| 15 "              |                   | $2.5 \times 10^5 \rightarrow 2.2 \times 10^6$ |
| 21 "              |                   | $3 \times 10^6 \rightarrow 4.6 \times 10^6$   |

Growth arrested:

|                   |                   |                   |
|-------------------|-------------------|-------------------|
| 3 hrs             | $8.9 \times 10^3$ | $4 \times 10^4$   |
| 1 day             | $6.1 \times 10^4$ | $4.4 \times 10^4$ |
| 4 "               | $6.2 \times 10^5$ | $4 \times 10^4$   |
| 8 " $\rightarrow$ | $1.3 \times 10^6$ | $5 \times 10^4$   |
| 10 "              | $9.2 \times 10^6$ | $5.2 \times 10^4$ |
| 15 -              |                   | $1 \times 10^4$   |
| 21                | $5 \times 10^5$   | $1.6 \times 10^4$ |
|                   |                   | $1.4 \times 10^4$ |
|                   |                   | $2.2 \times 10^4$ |

$= 8 \times 10^3$

#4  
Mohan  
World Bank

EXHIBIT

238



## **EXHIBIT K**

7/12

① harvest  
flasks p150

ad 1  
ad 11  
Bailey

② freeze: 11-Q - ⑥  
② 11-N - ⑥ 11-N - ③  
11-M - ③

split into (p100)

As49 m12 hygro (freeze)

m56-CrmA ② (Freeze + split) (p100)

11.6.11 (split 1:3) just a bit too heavy

CrmA - 11.6 ⑤ split / freeze

11.6-CrmA ⑨ split / freeze

Bring up m56-CrmA<sub>2</sub> to FLASK

3 Harvest virus spinner

4 split other spinners

My Spinner:

-  $5.4 \times 10^5$  cells / ml  
split 1/2 - freeze cells

-  $4.4 \times 10^5$  / ml / 1 liter removed  
and frozen

EXHIBIT

K1

8 - 100MM

7/13

todo

phone:

Ab in White / red File on Arms deck

Inventory virus bank \* look / + check

✓ (KBS from freezer) / when brought up

$2 \times 10^5 / ml$

Develop the gel (Monday)

$7.5 \times 10^7$

✓ (Cells frozen + thrown out)

no less than  $2 \times 10^5$   
3mls / 200mls cell

(Losses / media)

Ad 1 + Ad 6 Virus stock (5mls of stock)

Set up  
1-100ml → stock

grow up 11.6 pre-Absorbed. ✓ set up

293 cells from Kaye (Diplo In.) of  
Ginsberg: H5N1 III (earliest stock) (175 flasks)

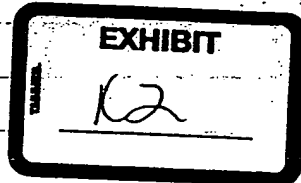
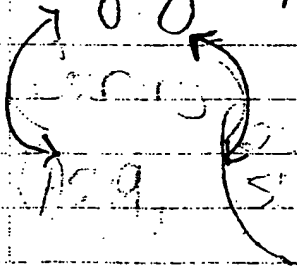
V5103 S-2-1 stock 50ml → 100mm plate → flasks

V5122 S-4-1 spin down / media 20% FBS 100ml → 100mm plate

(harvested) (9.60725)

Bayley 11/07/6 (911003) 104-8 loc 50ml → 100mm plate

freeze / thaw  
harvested (9.60725) 104-8



1. spinner 1 liter K.B.'s

Count  $3.4 \times 10^5$  cells/ml  
split 500ml into a 3 liter flask add 1.5 liter  
Joklik's media. Bring original 1 liter spinner  
up to 1 liter (Joklik's + 10% horse serum)

2. 3 liter spinner of K.B.'s infected

Count is  $3.2 \times 10^5$  cells/ml  
~~use~~ infect with (Bailey) 11/07/6 9/1003)

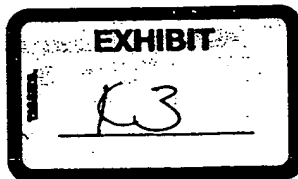
Bailey used was of a new stock grown up  
in 293 cells. last week Bailey. 9600;  
(Titer unknown) infected at 11:30 → 12:30

used 20ml of virus into 1 liter of cells  
for 1 hour. then added back media  
(2 liters Joklik's + 100 ml denatured  
Horse serum)

3. develop gels.

4. split 293 give one plate to Ann. (split other cells)

5. look at plaques that are forming.  
very few now since yesterday  
count tomorrow.



1. Split 293, A549 in 60mm (dishes)
2. Bring 3 liter spinner up to add 1 liter  
Count.  $4.2 \times 10^5$

infect tomorrow w/ (704)

3. New media into all 56-29, 56-25, MSB-2MD, MSB-3MD, CMA-56-③, 11.6-1MD, 11.6 CMA-② + A54911-Q

4. Harvest infected spinner; Bailey, wash pellet w/ PBS 2x (cold) resuspend it into 24mls total vol of .01 M Tris pH 8.0  
mls of cells + Tris = 24mls  
put into snap cap tubes & freeze at ~~200~~ -70°.  
put in freeze (right inside Lindas lab)

5. split & freeze ~~200~~ - 500mls of KB back up spinner.

Backup

6. Cells are not completely infected some are dying so must harvest cells

EXHIBIT

K4

C&C Banded the virus Ad1 960825  
1101/1107 Barleys (960802)

1 freeze (thaw)  
2 sonicate X 2  
3 Vol after son 1101 < 21mls

Ad1 < 19mls

add  $V \times 0.51 = g$  of GCL

10.71g in 1101/1107 Barleys  
9.69g w Ad1.

infect 3 liter spinner with  
dl 717 Vp 891208 Titer  $1.3 \times 10^{11}$

cell count 3 liter  $3.5 \times 10^5$   
Vol = 3 liters (3000mls)

$3.5 \times 10^5 \times 3000\text{mls} = 10.5 \times 10^8$  total cells

use 20 pfals / cell

$\frac{1.62 \text{ ul} \times 10^{11}}{1.3 \times 10^{11}} \times 20 \text{ pfals}$

KB's are healthy no clumping

EXHIBIT

K5

Black (first)

| 960 |                                |                      | 9/25  | Plaque Assay |   |                                    | Comment |
|-----|--------------------------------|----------------------|-------|--------------|---|------------------------------------|---------|
|     | Uruso                          | Dil                  | A     | B            | C |                                    |         |
| 1.  | 1101/1107<br>(first infection) | $.5 \times 10^{-6}$  | 9     | 25           | — |                                    |         |
|     |                                | $.5 \times 10^{-7}$  | 3     | 1            | — |                                    |         |
|     |                                | $.5 \times 10^{-8}$  | Ø     | Ø            | Ø |                                    |         |
|     |                                | $.5 \times 10^{-9}$  | Ø     | Ø            | Ø |                                    |         |
|     |                                | $.5 \times 10^{-10}$ | Ø     | Ø            | Ø |                                    |         |
| 2   | 765                            | $.5 \times 10^{-6}$  | TMTCA |              |   |                                    |         |
|     |                                | $.5 \times 10^{-8}$  | 3     | Ø            | Ø |                                    |         |
|     |                                | $.5 \times 10^{-9}$  | Ø     | Ø            | Ø |                                    |         |
|     |                                | $.5 \times 10^{-10}$ | Ø     | Ø            | Ø |                                    |         |
| 3.  | 717                            | $.5 \times 10^{-8}$  | Ø     |              |   | Very hard to see<br>plaques. today |         |
|     |                                | $.5 \times 10^{-9}$  | Ø     | Ø            | Ø |                                    |         |
|     |                                | $.5 \times 10^{-10}$ | Ø     | Ø            | Ø |                                    |         |

EXHIBIT  
K6

EXHIBIT

K6

| <div>1101 / 1107</div> <div> <math>.5 \times 10^6</math><br/> <math>.5 \times 10^7</math><br/> <math>.5 \times 10^8</math><br/> <math>.5 \times 10^9</math><br/> <math>.5 \times 10^{10}</math> </div>  | <div>A</div> <div> TMTCA<br/> 42<br/> 6<br/> 1<br/> 0 </div>                            | <div>B</div> <div> TMTCA<br/> 53<br/> 4<br/> 0<br/> <del>28</del> </div> | <div>C</div> <div> 8<br/> 1<br/> 0 </div>    | <div>Comments</div> <div>small plgs. (flat)</div>                                    |
|---|---|--|--|--|
| <div>717</div> <div> <math>.5 \times 10^6</math><br/> <math>.5 \times 10^8</math><br/> <math>.5 \times 10^9</math><br/> <math>.5 \times 10^{10}</math> </div> <div>765</div> <div> <math>.5 \times 10^6</math><br/> <math>.5 \times 10^8</math><br/> <math>.5 \times 10^9</math><br/> <math>.5 \times 10^{10}</math> </div> | <div> TMTCA<br/> TMTCA ←<br/> 34<br/> 1 </div> <div> TMTCA<br/> 12<br/> 2<br/> 0 </div> | <div> 28<br/> 3 </div> <div> 19<br/> 1<br/> 0 </div>                     | <div> 36<br/> 3 </div> <div> 1<br/> 0 </div> | <div> hard to count<br/> because everywhere<br/> (very faint)<br/> very small </div> |
|   |   |  |  |  |

EXHIBIT

K7



1101/1107

$1.5 \times 10^6$   
 $1.5 \times 10^{-7}$   
 $1.5 \times 10^8$   
 $1.5 \times 10^9$   
 $1.5 \times 10^{10}$

A  
 TMTZ  
 9  
 14  
~~8~~  
~~8~~

B  
 TMTZ  
 14  
 14  
~~8~~  
 1

C  
 12  
~~8~~  
~~8~~

Comment  
 start off small  
 grow very  
 quickly

717

$1.5 \times 10^{-6}$   
 $1.5 \times 10^{-8}$   
 $1.5 \times 10^{-9}$   
 $1.5 \times 10^{-10}$

TMTZ  
 TMTZ  
 59  
 4

45  
 4

55  
 8

Very very  
 small

765-

$1.5 \times 10^{-6}$   
 $1.5 \times 10^{-8}$   
 $1.5 \times 10^{-9}$   
 $1.5 \times 10^{-10}$

TMTZ  
 9  
 2  
 1

11  
 3  
~~8~~

3 monolayer  
 strong

EXHIBIT

18

| <div>5</div> <div>1101/1107</div> <div> <math>1.5 \times 10^6</math><br/> <math>.5 \times 10^{-7}</math><br/> <math>.5 \times 10^8</math><br/> <math>.5 \times 10^9</math><br/> <math>.5 \times 10^{10}</math> </div> | <div>A</div> <div>           TmTC<br/>           all together had<br/>           5<br/> <math>\emptyset</math><br/> <math>\emptyset</math> </div> | <div>B</div> <div>           mold + mTC<br/>           to count<br/>           7<br/> <math>\emptyset</math><br/> <math>\emptyset</math> </div> | <div>C</div> <div>           2<br/> <math>\emptyset</math><br/> <math>\emptyset</math> </div> | <div>Comment</div>           |
|---|---|---|---|------------------------------|
| <div>717</div> <div> <math>1.5 \times 10^6</math><br/> <math>.5 \times 10^8</math><br/> <math>1.5 \times 10^9</math><br/> <math>.5 \times 10^{10}</math> </div>   | <div>           TmTC<br/>           TmTC<br/>           14<br/>           5         </div>  | <div>           —<br/>           TmTC<br/>           13<br/>           4         </div>   | <div>           —<br/>           7<br/>           10         </div>                           | <div>small but visible</div> |
| <div>765</div> <div> <math>1.5 \times 10^6</math><br/> <math>1.5 \times 10^8</math><br/> <math>1.5 \times 10^9</math><br/> <math>1.5 \times 10^{10}</math> </div>   | <div>           TmTC<br/>           5<br/>           1<br/>           1         </div>  | <div>           3<br/>           2<br/>           mold         </div>   | <div>           3<br/>           1         </div>   |                              |

0

EXHIBIT  
 K9

1101/1107

$1.5 \times 10^6$   
 $1.5 \times 10^{-7}$   
 $1.5 \times 10^8$   
 $1.5 \times 10^9$   
 $1.5 \times 10^{10}$

A

Tmrc  
 Tmrc  
 no new aed  
 $\emptyset$   
 $\emptyset$

B

Tmrc  
 $\emptyset$   
 $\emptyset$   
 $\emptyset$

C

Tmrc  
 $\emptyset$   
 $\emptyset$

Comment

717

$1.5 \times 10^6$   
 $1.5 \times 10^8$   
 $1.5 \times 10^9$   
 $1.5 \times 10^{10}$

Tmrc  
 Tmrc  
 Tmrc  
 3

Tmrc  
 Tmrc  
 $\emptyset$

Tmrc  
 2

765

$1.5 \times 10^6$   
 $1.5 \times 10^8$   
 $1.5 \times 10^9$   
 $1.5 \times 10^{10}$

Tmrc  
 $\emptyset$   
 $\emptyset$   
 $\emptyset$

2  
 $\emptyset$   
 throw out

mono layer  
 gene  
 $\emptyset$   
 $\emptyset$

EXHIBIT

K10

1101/1107

$1.5 \times 10^6$   
 $.5 \times 10^{-7}$   
 $.5 \times 10^8$   
 $.5 \times 10^9$   
 $.5 \times 10^{10}$

| A   | B                                      | C                                 | Comment                                      |
|---|--|-----------------------------------|--|
| TMTCA<br>7<br>3<br><del>0</del><br><del>0</del> | 2<br>5<br><del>0</del><br><del>0</del> | 6<br><del>0</del><br><del>0</del> | plg camp<br>up squawly<br>taken taped<br>off |

717

$1.5 \times 10^{-6}$   
 $.5 \times 10^{-8}$   
 $1.5 \times 10^{-9}$   
 $.5 \times 10^{-10}$

|                         |                 |         |
|-------------------------|-----------------|---------|
| TMTA<br>TMTA<br>16<br>3 | TMTA<br>17<br>4 | 19<br>7 |
|-------------------------|-----------------|---------|

765

$1.5 \times 10^{-6}$   
 $1.5 \times 10^{-8}$   
 $1.5 \times 10^{-9}$   
 $1.5 \times 10^{-10}$

|                                 |                                   |                              |
|---------------------------------|-----------------------------------|------------------------------|
| TMTCA<br>5<br>1<br><del>0</del> | 3<br><del>0</del><br><del>0</del> | <del>0</del><br><del>0</del> |
|---------------------------------|-----------------------------------|------------------------------|

EXHIBIT

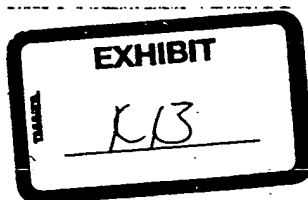
K11

|                      | A    | B            | C  | Comment |
|----------------------|------|--------------|----|---------|
| 1101/1107            |      |              |    |         |
| $1.5 \times 10^6$    | TMTc | TMTc         | -  |         |
| $1.5 \times 10^{-7}$ | TMTc | TMTc         | -  |         |
| $1.5 \times 10^8$    | +1   | +2           | +0 |         |
| $1.5 \times 10^9$    | +0   | 0            | +0 |         |
| $1.5 \times 10^{10}$ | 0    | <del>X</del> | 0  |         |
| 717                  |      |              |    |         |
| $1.5 \times 10^6$    | TMTc | -            | -  |         |
| $1.5 \times 10^8$    | TMTc | -            | -  |         |
| $1.5 \times 10^9$    | TMTc | -            | -  |         |
| $1.5 \times 10^{10}$ | +0   | +2           | +0 |         |
| 765                  |      |              |    |         |
| $1.5 \times 10^6$    | TMTc | -            | -  |         |
| $1.5 \times 10^8$    | +1   | +1           | -  |         |
| $1.5 \times 10^9$    | +0   | +0           | +0 |         |
| $1.5 \times 10^{10}$ | +1   | +0           | -  |         |

EXHIBIT

K12

|                      | A           | B                     | C           | Comment             |
|----------------------|-------------|-----------------------|-------------|---------------------|
| 1101/1107            |             |                       |             |                     |
| $1.5 \times 10^6$    | mold        | 34                    |             |                     |
| $1.5 \times 10^{-7}$ | thru        | blended together thru |             |                     |
| $1.5 \times 10^8$    | 5           | 4                     | 9           | Really big          |
| $1.5 \times 10^9$    | $\emptyset$ | $\emptyset$           | $\emptyset$ |                     |
| $1.5 \times 10^{10}$ | $\emptyset$ | $\emptyset$           | $\emptyset$ |                     |
| 765                  |             |                       |             |                     |
| $1.5 \times 10^6$    | TMT         |                       |             |                     |
| $1.5 \times 10^8$    | TMT         |                       |             |                     |
| $1.5 \times 10^9$    | 4           | 3                     | 12          | monolayer looks bad |
| $1.5 \times 10^{10}$ | $\emptyset$ | $\emptyset$           | $\emptyset$ | no new just bigger  |
| 768                  |             |                       |             |                     |
| $1.5 \times 10^6$    | TMT         | TMT                   |             |                     |
| $1.5 \times 10^8$    | TMT         | TMT                   |             |                     |
| $1.5 \times 10^9$    | TMT         | TMT                   |             |                     |
| $1.5 \times 10^{10}$ | 3           | $\emptyset$           | 2           |                     |



|           |   | A                               | B                               | C               | comment |
|-----------|---|---------------------------------|---------------------------------|-----------------|---------|
| 760       | $5 \times 10^{-8}$<br>$5 \times 10^{-9}$<br>$5 \times 10^{-10}$   | TMTZ<br>37<br>3                 | TMTZ<br>29<br>1                 | TMTZ<br>26<br>4 |         |
| 327       | $5 \times 10^{-8}$<br>$5 \times 10^{-9}$<br>$5 \times 10^{-10}$   | TMTZ<br>60<br>18                | TMTZ<br>74<br>17                | 74              |         |
| 1101/1107 | $5 \times 10^{-6}$<br>$5 \times 10^{-7}$<br>$5 \times 10^{-8}$<br>$5 \times 10^{-9}$<br>$5 \times 10^{-10}$ | TMTZ<br>TMTZ<br>TMTZ<br>66<br>8 | TMTZ<br>TMTZ<br>TMTZ<br>64<br>7 | 70              | Bg Alp  |
| 702       | $5 \times 10^{-8}$<br>$5 \times 10^{-9}$<br>$5 \times 10^{-10}$   | TMTZ<br>TMTZ<br>36              | TMTZ<br>TMTZ<br>30              | TMTZ<br>36      |         |



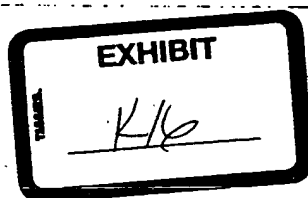
|           |  | A   | B                | C  | comment              |
|-----------|--|---|------------------|--|----------------------|
| 760       | $.5 \times 10^{-8}$<br>$.5 \times 10^{-9}$<br>$.5 \times 10^{-10}$   | TmTC<br>44<br>5   | TmTC<br>40<br>4  | TmTC<br>42<br>6                            |                      |
| 327       | $.5 \times 10^{-8}$<br>$.5 \times 10^{-9}$<br>$.5 \times 10^{-10}$   | TmTC<br>64 <i>really</i><br>18 <i>two</i>               | TmTC<br>74<br>16 | 72<br>37 <i>Missed counting first time</i> |                      |
| 1101/1102 | $.5 \times 10^{-6}$<br>$.5 \times 10^{-7}$<br>$.5 \times 10^{-8}$<br>$.5 \times 10^{-9}$<br>$.5 \times 10^{-10}$ | TmTC<br>TmTC<br>TmTC<br>25<br>11                        | <hr/> 21<br>9    | <hr/> 22                                   | <i>mid nice plg.</i> |
| 702       | $.5 \times 10^{-8}$<br>$.5 \times 10^{-9}$<br>$.5 \times 10^{-10}$   | TmTC<br><del>almost</del> <i>almost</i><br>15 <i>21</i> | TmTC<br>24<br>14 | 22<br>15                                   |                      |

EXHIBIT

K15

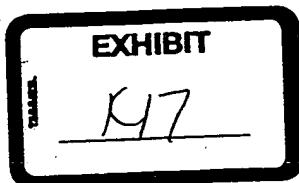


|           |   | A                              | B                           | C                 | Comment |
|-----------|---|--------------------------------|-----------------------------|-------------------|---------|
| 760       | $5 \times 10^{-8}$<br>$5 \times 10^{-9}$<br>$5 \times 10^{-10}$   | TMTc<br>10<br>1                | TMTc<br>20<br>3             | TMTc<br>15<br>1   | small   |
| 327       | $5 \times 10^{-8}$<br>$5 \times 10^{-9}$<br>$5 \times 10^{-10}$   | TMTc<br>TMTc<br>10             | TMTc<br>TMTc<br>8           | TMTc<br>TMTc<br>9 |         |
| 1101/1102 | $5 \times 10^{-6}$<br>$5 \times 10^{-7}$<br>$5 \times 10^{-8}$<br>$5 \times 10^{-9}$<br>$5 \times 10^{-10}$ | TMTc<br>TMTc<br>TMTc<br>4<br>0 | —<br>TMTc<br>TMTc<br>5<br>0 | 4<br>—            |         |
| 702       | $5 \times 10^{-8}$<br>$5 \times 10^{-9}$<br>$5 \times 10^{-10}$   | TMTc<br>TMTc<br>3              | TMTc<br>3                   | 5                 |         |



2m/min

|           |   | A                                 | B                        | C          | Comments                  |
|-----------|---|-----------------------------------|--------------------------|------------|---------------------------|
| 760       | $1.5 \times 10^{-8}$<br>$1.5 \times 10^{-9}$<br>$1.5 \times 10^{-10}$   | TMTCA<br>TMTCA<br>4               | TMTCA<br>TMTCA<br>3      | 3<br>1     | Very small easy to calib. |
| 327       | $1.5 \times 10^{-8}$<br>$1.5 \times 10^{-9}$<br>$1.5 \times 10^{-10}$   | TMTCA<br>TMTCA<br>3               | TMTCA<br>TMTCA<br>5      | TMTCA<br>6 |                           |
| 1101/1107 | $1.5 \times 10^{-6}$<br>$1.5 \times 10^{-7}$<br>$1.5 \times 10^{-8}$<br>$1.5 \times 10^{-9}$<br>$1.5 \times 10^{-10}$ | TMTCA<br>TMTCA<br>TMTCA<br>0<br>2 | TMTCA<br>TMTCA<br>1<br>1 | 0<br>0     | Very acidic               |
| 702       | $1.5 \times 10^{-8}$<br>$1.5 \times 10^{-9}$<br>$1.5 \times 10^{-10}$   | TMTCA<br>TMTCA<br>1               | TMTCA<br>0               | TMTCA<br>1 | Very close hard to count  |



760

$1.5 \times 10^{-8}$   
 $1.5 \times 10^{-9}$   
 $1.5 \times 10^{-10}$

TMTCA  
TMTCA  
3

TMTCA  
TMTCA  
2

2

common

327

$1.5 \times 10^{-8}$   
 $1.5 \times 10^{-9}$   
 $1.5 \times 10^{-10}$

TMTCA  
TMTCA  
8

TMTCA  
TMTCA  
5

TMTCA  
2

1101/1107

$1.5 \times 10^{-6}$   
 $1.5 \times 10^{-7}$   
 $1.5 \times 10^{-8}$   
 $1.5 \times 10^{-9}$   
 $1.5 \times 10^{-10}$

$\emptyset$   $\emptyset$   $\emptyset$

all plates too  
acid no  
new colonies

702

$1.5 \times 10^{-8}$   
 $1.5 \times 10^{-9}$   
 $1.5 \times 10^{-10}$

TMTCA (throw out)  
TMTCA  
 $\emptyset$

TMTCA  
 $\emptyset$

TMTCA  
 $\emptyset$

throw  
out

Keep plates  $5 \times 10^{-10}$  760, 327  
still growing plga

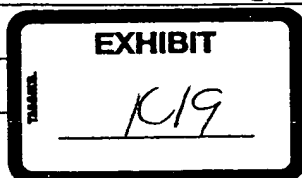
EXHIBIT  
K18

|   |           | Plaque Assays (% of final plaque #) |      |      |      |      |      |      |        |
|---|-----------|-------------------------------------|------|------|------|------|------|------|--------|
|   | Virus     | 5d                                  | 7d   | 12d  | 13d  | 16d  | 19d  | 25d  | 30d    |
| ① | 1101/1107 | 0.                                  | 18.3 | 55.  | 67.9 | 80.7 | 80.7 | 83.5 | 100.9% |
| ② | sub 765   | 2.6                                 | 32.8 | 57.8 | 65.5 | 79.3 | 81.  | 83.6 | 100.   |
| ③ | dl 717    | 0.                                  | 10.3 | 33.8 | 54.4 | 82.4 | 89.7 | 92.6 | 100.   |

titer of 1101/1107 :  $6.61 \times 10^9$  /ml  
(harvested from KB cells at day 2)

|   |              | 10-15-96 Plaque Assays (% of final plaque #) |      |      |      |        |
|---|--------------|--|------|------|------|--------|
|   | Virus        | 8d   | 10d  | 13d  | 16d  | 21d    |
| ① | pm 760       | 32.4   | 78.  | 94.2 | 97.7 | 100.7% |
| ② | dl 327       | 32.1   | 65.4 | 82.1 | 90.7 | 100.   |
| ③ | dl 1101/1107 | 65.7   | 94.6 | 98.7 | 100. | 100.   |
| ④ | dl 702       | 64.2   | 91.8 | 98.7 | 100. | 100.   |

titer of 1101/1107 :  $1.95 \times 10^{11}$  pfu/ml  
(harvested from KB cells at day 3)



|     | VIRUS                | From      | INF    | Harvest  | Spin | plaq | Comments                 | Freezer |
|-----|----------------------|-----------|--------|----------|------|------|--------------------------|---------|
| 1.  | Ad4A9K               | (VS58)    | 961016 | 961018 ✓ |      |      |                          |         |
| 2.  | Ad4AE <sub>3</sub>   | (961033)  | 961104 | 961106   |      |      |                          |         |
| 3.  | Ad4AE <sub>3</sub> B | (VS56)    | 961031 | 961102   |      |      |                          |         |
| 4.  | 1101/1107            | (Bailey)  | 960905 | 960907   | ✓    | ✓    |                          | 1-1     |
| 5.  | 1101/1107            | 2nd batch | 960917 | 960919   | ✓    | ✓    |                          | 1-1     |
| 6.  | d1702                | (VS33)    | 961002 | V2day    | ✓    | ✓    |                          | 1-2     |
| 7.  | d1773                | (VS230)   | 960909 | V2day    | ✓    |      |                          | 101-5   |
| 8.  | d1717                | (VS118)   | 960906 | 960908   | ✓    | ✓    |                          | 2-2     |
| 9.  | 327                  | (VS212)   | 960914 | 960917   | ✓    | ✓    |                          | 5-5     |
| 10. | Ad760                | (VS227)   | 960915 | 960917   | ✓    | ✓    |                          | 4-6     |
| 11. | Ad5                  | (VS198)   | 960907 | 960908   | ✓    | ✓    | Great bands              | 101-2   |
| 12. | Ad1                  | (sup)     | 960905 | 960907   | ✓    | ✓    |                          | 2-1     |
| 13. | d1762                | (Repeat)  | 960908 | 960908   | ✓    | ✓    | Bad no plaques           | 2-3     |
| 14. | Ad11                 | Sup       | 961030 | 961002   |      |      |                          |         |
| 15. | dL797                | (VS243)   | 960922 | 960924   |      |      |                          |         |
| 16. | Ad3                  |           |        |          |      |      | first spin               |         |
| 17. | Ad6                  | SUP       | 960801 | 960803   | ✓    | ✓    | Good bands               | 101-2   |
| 18. | Ad9                  | (sup)     | 970209 |          |      |      |                          |         |
| 19. | dL703                | VS50      | 961001 | 961003   | ✓    |      |                          |         |
| 20. | dL704                | (VS167)   | 960802 | 960804   | ✓    | ✓    | thin bands               | 3-2     |
| 21. | d1748                | (VS181)   | 960708 | 960810   | ✓    | ✓    |                          | 13-6    |
| 22. | d1798                | (VS188)   | 960708 | 960710   | ✓    | ✓    | Good                     | 13-8    |
| 23. | d1763                | (VS214)   | 960703 | 960705   | ✓    | ✓    | Bad Bands look good      | 2-3     |
| 24. | d1765                | (VS225)   | 960824 | 960826   | ✓    | ✓    |                          | 101-8   |
| 25. | d1739                | (VS223)   | 921023 | just plq | ✓    | ✓    |                          |         |
| 26. | d1754                | (VS243)   | 946202 | just plq | ✓    | ✓    |                          |         |
| 27. | d1712                | ?         | 950503 | —        | ✓    | ✓    | Plays Good               |         |
| 28. | 7001                 | ?         | 960724 | 960726   | ✓    | ✓    |                          |         |
| 29. | d1250                | sup       | 950616 | 950618   | ✓    | ✓    | Big plaques / nice bands |         |
| 30. | Ad41                 | sup/293   | (293)  | →        |      |      |                          |         |
| 31. | Ad40                 | sup/293   | (293)  | →        |      |      |                          |         |
| 32. | Ad707                | (VS212)   | 961008 | 961110   |      |      |                          |         |
| 33. | Ad740                | VS240     | 930612 |          |      |      |                          |         |
| 34. | d1762                | (VS243)   | 960920 | 961122   |      |      | From 640620 see page     |         |
| 35. | Ad15                 | 961024    | 970305 |          | ✓    |      |                          |         |
| 36. | Ad11                 |           | 970205 | 970205   | ✓    | ✓    | Great bands              |         |
| 37. | Ad11                 |           | 970205 | 970205   | ✓    | ✓    | Great " " !              |         |

EXHIBIT

K20

4/62  
Chris

How affinity Nerve growth receptor = .1 ug / ul

Viruses from Kostaya: grow in KB's first AB49  
Then KB.

= Viruses from Bowling = grow in AB49 first then  
then in KB! 2 flasks

Set up 2 flasks for Bowling virus. pme 116  
AAA

= KB's ready to infect in 3 liter spinner  
count

1 liter bring up to 1300 liters split  
tomorrow.

del 707:  $1.57 \times 10^{10}$   
count  $3.8 \times 10^5 \times 9.9 \times 10^8$  cells / liter  
$$\frac{0.99 \times 10^8}{1.57 \times 10^{10}} \times 10 \text{ PFU's} = 6.3 \text{ u}$$
$$\frac{0.99 \times 10^8}{1.57 \times 10^{10}} \times 5 \text{ PFU's} = 3.15$$

Infect:

EXHIBIT  
K21

4/28/monday

1. set up AS49 for virus from Colag
2. split MCF-7 for transfection of  
Nerve growth factor.
3. change media on MCF-7 - transfections  
11.6 - MCF-7 ~ 5-6 clones per dish  
MS6 - MCF-7 ~ 3-4 " "  
except for one dish which has a lot more

Trans:

set up transfection w/  
Gow Affinity Nerve growth factor

= 3 dishes  
+ 11.6 = 3 dishes  
2 controls

use new prep.

EXHIBIT

K22

Coverslips of mcf-7.

isob affinity Nerve growth factor .2 ug/ul  
use

Solution:

$A = (pK \log FRMN) \cdot 2 \text{ ug/ul} = 25 \text{ ul/dish}$   
NGF ONLY

3 NGF only

A = 75ul DNA + 800ul opti DMEM =

B = 36ul lip + 1300ul opti DMEM

A+B for 30min 2.4 ml opti

NGF + 11.6

5.7 11.6 + 75ul + 300ul opti DMEM

36ul lip + 300ul opti DMEM

A+B for 30min 2.4 ml opti

2 dishes not transfected = for control

- mix A+B let sit R.T for 30min, Add Reman-media
- 1ml per dish / for five hours,
- then add 20% media (1ml) let incubate O/N
- down tomorrow.

1 dish pmtz 2.2 + 100 opti DMEM

12 lipo + 100 opti DMEM

Add 1ml 20% on transfection / Add 2% to infection

Set up Plastro 293 (110 viruses)

Set up 4 A549 plates (100ul to pre absorb ab)

Add ~~for~~ Costas Virus to flasks - put split K B's

EXHIBIT

1623



5/2 Friday

- FREEZE FAS cells - pool
- V infection.
- split all cells /
- split KB's back

5/6

- split ASTA to two flasks - for Kostya
- clones picked
- infect the other // spinner //

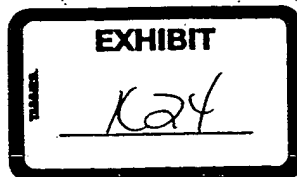
$4.5 \times 10^5$  cells/ml /  $\times 3000$  nls =

13,500 cells.

cells look ok: added 5 nls of  
the virus 45.5 from Kostya to  
the spinner

(Tomorrow pick clones.)  
get cells from Mohan  
make Jakkis media / ok

*mv*



1:30

5/13

797?



freeze + thaw + spn to CS prep

antigen

Zen

19mls

Zen

19mls

KD-1 1101/1107 ADP 970508

PME 11.6 AAA 970503

dl 707 970423

dl 751 970324

Viruses in warm room X2 thaw

~~antigen~~ also

Zen

dl 707 970315

7/2

2/1

split A549 cells to Fibro for 797 + others  
noted

IN Freezer need Sonicated in beater

location

PME = 1, 7

KD = 9, 3

751 = 11, 5

707 (970315) 4, 2

707 = 6, 12

set machine on  
4° / 35K for  
O/N ↔

EXHIBIT

K25

5/14

Viruses taken out of Centrifuge

(970508) <sup>(46-4)</sup> KD-1 (Kostya) 1101/1107 ADP = <sup>harvest</sup> ~ 4 mls (small thin bands)  
(970324) dl 751 (nice thick bands) good = ~ 4 mls  
(970315) dl 707 (very thick by bands great) = ~ 9 mls  
(970423) dl 707 (nice bands) good = ~ 6 mls

(970503) DME-11.6 AAA (Very nice bands) good ~ 5 mls / 3  
(BLY ADP-AAA)

(Note) Don't dilute KD-1 very much got alot of extra liquid w/ viruses band ~ 2 mls of actual virus.

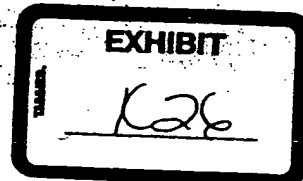
Dilute with TSB ~ 5x-10x

|  | tubes | LOC            |
|--|-------|----------------|
| KD-1 dilute bring up to (10 mls)                     | 5     | 4-4            |
| dl 751 bring up to (16 mls)                          | 8     | TRG 2-5<br>2-4 |
| dl 707 (970315) bring up to (24 mls)                 | 12    | 4-3<br>4-2     |
| dl 707 (970423) bring up to (16 mls)                 | 8     | 4-3            |
| DME-11.6 AAA = bring up to (14 mls)<br>BLY (ADP-AAA) | 7     | 4-4            |

(plaque assay viruses) 751 original 53-3/60-B  
one tube of each in my box to plaque 707 original

need to

|                   |           |                 |
|-------------------|-----------|-----------------|
| infect 3 liter w/ | HS dl 11  | sup from flasks |
| 3                 | HS dl 110 | AS49            |
|                   | dl 205    |                 |



5/20/97

ages assay. (Titer virus)

757 (970324)

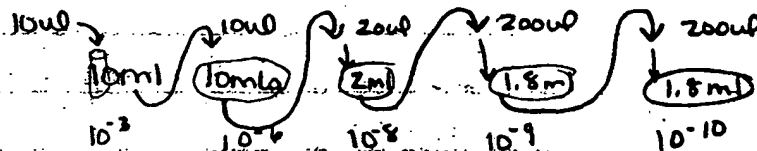
dl 707 (970315)

dl 707 (970423)

DME - 11.6 AAP (BLY-ADP-AAP) (970503)

KD-1 (970508) (Kostya)

dilute all viruses 10u0 to 10mls ( $10^{-3}$  dilution)



45  
205

2 -  $10^{-8}$

3 -  $10^{-9}$

3 -  $10^{-10}$

\* each

50

5

2

1

43

100 mls

2x DME

10 mls

Sodium Bi

4 mls

FBS

2 mls

Glut

86 mls

AGAR

Add virus to the 3liter spinner - dl 110 virus

3liter Count:  $4.2 \times 10^5$  cells/ml. use 1ml cells.

20110

1 liter Count:  $(2.7 \times 10^5 \text{ cells/ml})$  split.

EXHIBIT

K27

6/2

second 9/23

|                     | A    | B    | C  |
|---------------------|------|------|----|
| $5 \times 10^{-8}$  | TMTL | TMTL | /  |
| $5 \times 10^{-9}$  | 10   | bact | 10 |
| $5 \times 10^{-10}$ | 2    | 0    | 1  |

| A    | B    | C  |
|------|------|----|
| TMTL | TMTL |    |
| 9    | 3    | 10 |
| 3    | 0    | 0  |

707  
(11/10)  
11/8/11

|                     |    |    |   |
|---------------------|----|----|---|
| $5 \times 10^{-8}$  | 14 | 15 | / |
| $5 \times 10^{-9}$  | 2  | 5  | 2 |
| $5 \times 10^{-10}$ | 0  | 0  | 0 |

|    |    |   |
|----|----|---|
| 68 | 47 |   |
| 8  | 2  | 0 |
| 0  | 0  | 0 |

707

|                     |    |    |   |
|---------------------|----|----|---|
| $5 \times 10^{-8}$  | 13 | 15 | / |
| $5 \times 10^{-9}$  | 3  | 0  | 0 |
| $5 \times 10^{-10}$ | 0  | 0  | 3 |

|    |    |   |
|----|----|---|
| 30 | 17 |   |
| 2  | 3  | 0 |
| 0  | 0  | 0 |

KD-7

|                     |              |     |              |
|---------------------|--------------|-----|--------------|
| $5 \times 10^{-8}$  | Total<br>162 | 163 | Total<br>162 |
| $5 \times 10^{-9}$  | Total<br>46  | 49  | Total<br>43  |
| $5 \times 10^{-10}$ | 1            | 0   | 0            |

|      |                   |   |
|------|-------------------|---|
| TMTL | TMTL              |   |
| 3    | 1                 | 1 |
| 1    | 0                 | 2 |
|      | $8.1 \times 10^9$ |   |

PMF  
(in case prep)

|                     |    |    |    |
|---------------------|----|----|----|
| $5 \times 10^{-8}$  | 74 | 78 | /  |
| $5 \times 10^{-9}$  | 16 | 11 | 28 |
| $5 \times 10^{-10}$ | 4  | 3  | 2  |

|      |      |   |
|------|------|---|
| TMTL | TMTL |   |
| 12   | 14   | 7 |
| 1    | 0    | 2 |

EXHIBIT  
K28

W1 38 infections with 1101/1107 and d1308

11/24

MOI dividing quiescent 549

0.1

1

10

100

16 35 mm W1 38

8 35 mm AS49  $2.5 \times 10^5$  cells/dish

infections 11/25

AS49  $2.5 \times 10^5$  cells

$5 \times 10^4$  0.1

$5 \times 10^5$  1

$5 \times 10^6$  10

$5 \times 10^7$  100

MOI

$1.34 \times 10^7$   
1101/1107 (960317)  
 $3.8 \times 10^5$

$3.8 \times 10^2$   
10x dish 6ul

~~2.710<sup>3</sup>~~  
~~312~~  
~~3.8~~

change medium on W138 to 0.2% FCS

11/22 infect started W138 with

d308 (950511)

100

10

MOI

1

0.1

1101/1107 (960317)

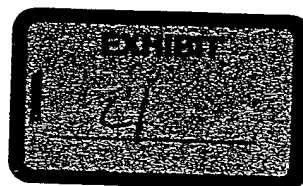
100

10

MOI

1

0.1



# ADP Western

infect AS41 with 303, KDI 12/9

$10^6$  cells / dish  $8.08 \times 10^6$  / ml  $303 \Rightarrow 8.08 \times 10^6$  / ml  
 $50 \mu\text{g}/\text{cell} \Rightarrow 6.8 \mu\text{l}$

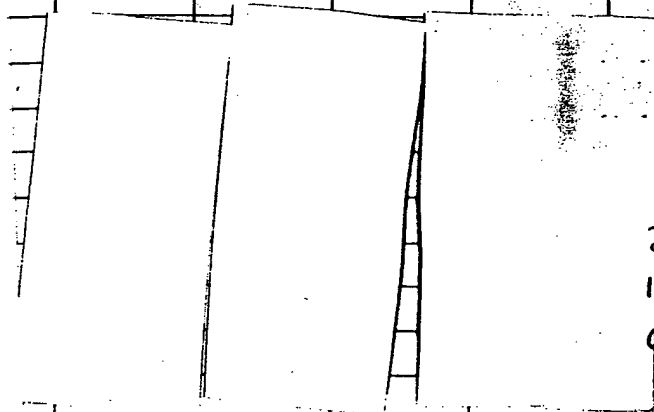
$6.12 \times 10^6$  / ml  $\text{KDI} \Rightarrow 6.12 \times 10^6$  / ml

$50 \mu\text{g}/\text{cell} \Rightarrow 8.16 \mu\text{l}$

Harvest 33h p.i.

|        |       |     |                   |
|--------|-------|-----|-------------------|
| $\phi$ | 0.190 |     |                   |
| mark   | 0.280 | 100 | 5 $\mu\text{l}$   |
| 303    | 0.359 | 173 | 2.8 $\mu\text{l}$ |
| KDI    | 0.320 | 140 | 3.6 $\mu\text{l}$ |

4



15  
5  
5  
5  
5  
5

10 and 15% SDS PAGE

[mark, 303, KDI] 3X, BR

hangover mark 12.5 10%, 4.56/5%

block 10% milk OK

cut 1st ab

4 12522II  
 5 12531  
 6 100578

1:400

Wash

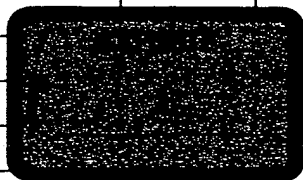
2nd ab

Wash

3X

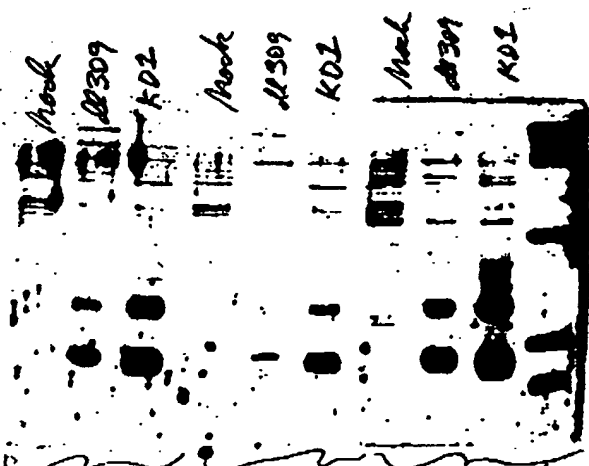
2 rabbit HKPO

3X





- KD1 expressed Ad5 Ab well  
 - Can detect ADP by Western  
 - Prelim - look like KD1 expresses more ADP than KD2?  
 Used 500 PFU of KD1  
 Used 50 PFU of KD2



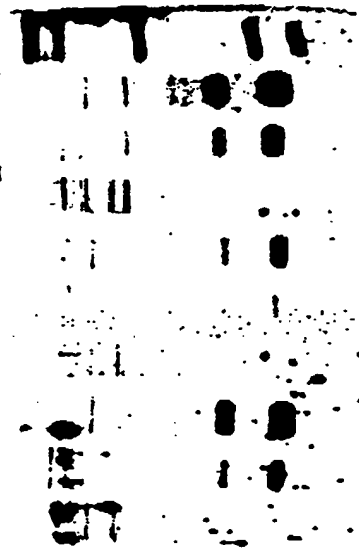
APP —  
 ADP —

3 diff antibodies

12532E  
 12531  
 100574  
 Use this

ADP Western  
 3d L per

Next exp - Time course  
 KD1  
 KD2  
 KD3  
 1101/1107





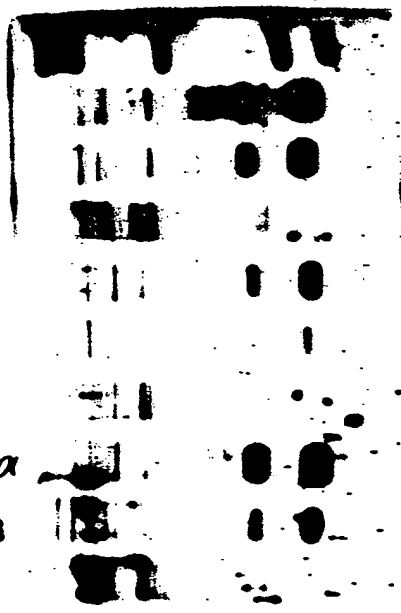
12/12/87



W01  
308  
mod

W01  
308  
mod

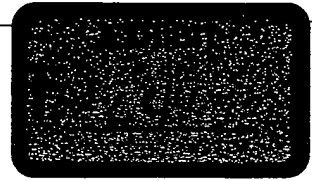
W01  
308  
mod

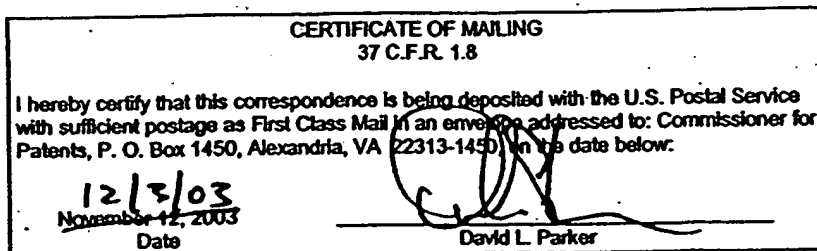


1005H

1252

1252





**PATENT**

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

In re Application of:  
Wold *et al.*

Serial No.: 09/351,778

Filed: July 12, 1999

For: Replication Competent Anti-Cancer Vectors

Group Art Unit: 1632

Examiner: Priebe, Scott David

Atty. Dkt. No.: 66153-7775

**SUPPLEMENTAL DECLARATION OF THE INVENTORS UNDER 37 C.F.R §1.131**

We, William S.M. Wold, Ann E. Tollefson, Konstantin Doronin and Karoly Toth, declare as follows:

1. We are joint inventors of the subject matter claimed in the referenced patent application. We are submitting the present §131 declaration to supplement our earlier declaration filed January 6, 2003, the contents of which are incorporated herein by reference.
2. In our earlier declaration we demonstrated that the adenovirus vector KD1 was constructed prior to March 3, 1997, and, in numerous studies that we conducted between 5/9/97 and 6/2/97, KD1 was shown to overexpress ADP. We are submitting the present Supplemental Declaration to supplement our earlier declarations by demonstrating that (1) prior to March 3, 1997, we conceived of using ADP-expressing adenoviral vectors for treating cancer in patients,

and that (2) shortly after March 3, 1997 an exemplary vector, KD1, was tested in an animal having cancer and shown to have anticancer activity.

3. Prior to March 3, 1997 we conceived of the idea of using adenovirus vectors expressing the ADP gene as a therapeutic agent to treat cancer. This is shown, for example, in the exhibits to our 1/6/03 declaration, in particular, Exhibit B at, for example, page 3, section B, and pages 4-8. The Exhibit B document is dated prior to March 3, 1997.

4. On July 7, 1997, we sent KD1, dl1101/1107, dl309, and A549 cells to Dr. Jeffrey A. Whitsett at the Children's Hospital Medical Center, Division of Pulmonary Biology, Cincinnati, OH. A copy of the cover letter sent to Dr. Whitsett is attached as Exhibit M. Dr. Whitsett had agreed to test these vectors on our behalf in the A549 nude mouse model, in which A549 human lung carcinoma cells are used to establish tumors in nude mice following by injection of the various vectors to determine their anticancer efficacy. On September 16, 1997, we received a report from Dr. Whitsett's colleague, Lee Zhang, indicating that "10<sup>9</sup> pfu of each of the viruses were injected into each established A549 tumor. 4 out of 6 tumors injected with KD1 showed slowed tumor growth while 2 out of 2 tumors injected with dl309 and 4 out of 4 injected with dl 1101/1107 continued to grow." A copy of this fax is attached as Exhibit N.

5. Accordingly, foregoing studies conducted on our behalf demonstrated the successful use of KD1 in the treatment of cancer in an animal at least as early as September, 1997.

6. We hereby declare that all statements made of our own knowledge are true and all statements made on information are believed to be true and further that the statements were made with the knowledge that willful false statements and the like so made are punishable by fine or

imprisonment or both under § 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of this application or any patent issued thereon.

December 1, 2003  
Date

William S.M. Wold  
William S.M. Wold

December 1, 2003  
Date

Ann E. Tollefson  
Ann E. Tollefson

December 1, 2003  
Date

Konstantin Doronin  
Konstantin Doronin

December 1, 2003  
Date

Karoly Toth  
Karoly Toth



SAINT LOUIS UNIVERSITY  
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Department of Molecular  
Microbiology & Immunology

William S. M. Wold, Ph.D.  
Professor and Chairman

7/8/97

Dear Jeff:

- Please find enclosed:

ΔΔ309,  $2.15 \times 10^8$  PFU/ml, ~ 200  $\mu$ l

ΔΔ1101/1107,  $1.95 \times 10^8$  PFU/ml, 2 ml

KD2,  $9 \times 10^{10}$  PFU/ml, 2 ml

A549 cells, passage 78.

- ΔΔ309 is Ad5, with the E3 10.4k, 14.5k, 14.7k genes <sup>deleted</sup>
- ΔΔ1101/1107, deletes the RB and p300 binding sites required to drive cells from G<sub>0</sub>→S phase, in ΔΔ309 <sup>background</sup>
- KD2, is in ΔΔ1101/1107 background.  
Lacks all E3 (stealth) genes. Engineered to overexpress the Adenovirus Death Protein (ADP).

- Bill

## **EXHIBIT N**

FAX MESSAGE

DATE 9/16/97

TO

Name Bill Wald

Address

Fax Number 314 713 3403 Phone Number

Number of pages (including this one) 1

MESSAGE: 10<sup>7</sup> pfu of adenovirus were injected into each  
established R49 tumor. 4 out of 6 tumors injected  
with KD1 showed altered tumor growth. While 2 out of  
9 tumors injected with dl309 and 4 out of 4 injected  
with dl u01/u07 continued to grow.

FROM

Name Lei Zhang

CHILDREN'S HOSPITAL MEDICAL CENTER

DIVISION OF NEONATOLOGY

3333 Burnet Avenue

Cincinnati, Ohio 45229-3039

Phone (513) 636-7892

Fax (513) 636-7868

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# **TERMINAL DISCLAIMER TO OBIUATE A DOUBLE PATENTING REJECTION OVER A PRIOR PATENT**

Docket Number (Optional)  
INGN:109US

In re Application of: William S. M. Wold, et al.

Application No.: 09/351,778

Filed: July 12, 1999

For: REPLICATION COMPETENT ANTI-CANCER VECTORS

The owner\*, Saint Louis University, of 100 percent interest in the instant application hereby disclaims, except as provided below, the terminal part of the statutory term of any patent granted on the instant application, which would extend beyond the expiration date of the full statutory term defined in 35 U.S.C. 154 and 173, as presently shortened by any terminal disclaimer, of prior Patent No. 6,627,190. The owner hereby agrees that any patent so granted on the instant application shall be enforceable only for and during such period that it and the prior patent are commonly owned. This agreement runs with any patent granted on the instant application and is binding upon the grantee, its successors or assigns.

In making the above disclaimer, the owner does not disclaim the terminal part of any patent granted on the instant application that would extend to the expiration date of the full statutory term as defined in 35 U.S.C. 154 and 173 of the prior patent, as presently shortened by any terminal disclaimer, in the event that it later: expires for failure to pay a maintenance fee, is held unenforceable, is found invalid by a court of competent jurisdiction, is statutorily disclaimed in whole or terminally disclaimed under 37 CFR 1.321, has all claims canceled by a reexamination certificate, is reissued, or is in any manner terminated prior to the expiration of its full statutory term as presently shortened by any terminal disclaimer.

Check either box 1 or 2 below, if appropriate.

1. ☐ For submissions on behalf of an organization (e.g., corporation, partnership, university, government agency, etc.), the undersigned is empowered to act on behalf of the organization.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

2. ☒ The undersigned is an attorney or agent of record.

Monica A. De La Paz 11/03/2004  
Signature Date

Monica A. De La Paz, Reg. No. 54,662

Typed or printed name

(512) 536-5639

Telephone Number

- ☒ Terminal disclaimer fee under 37 CFR 1.20(d) included.

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This collection of information is required by 37 CFR 1.321. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

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